HANDBOOK OF ANTISEPTICS

DAKIN & DUNHAM

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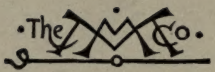
A HANDBOOK OF ANTISEPTICS



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A HANDBOOK

OF

ANTISEPTICS

BY

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LOUIS PASTEUR
1822–1895

JOSEPH LISTER
1827–1913

An Extract from Pasteur's Address at the Celebration of his Seventieth Birthday

You lastly, delegates of foreign nations, who have come from so far to give proof of your sympathy with France — you bring me the deepest happiness which a man can experience who believes implicitly that science and peace will triumph over ignorance and war, that people will learn to agree together, not for purposes of destruction but for improvement, and that the future will belong to those who shall have done the most for suffering humanity.

I address myself to you, my dear Lister, and to all of you illustrious representatives of Science, of Medicine, and of Surgery.

Young people, young people, confine yourselves to those methods, sure and powerful, of which we as yet know only the first secrets. And all, however noble your career, never permit yourselves to be overcome by scepticism, both unworthy and barren; neither permit the hours of sadness which pass over a nation to discourage you. Live in the serene peace of your laboratories and your libraries. First ask yourselves, What have I done for my education? then, as you advance in life, What have I done for my country? so that some day that supreme happiness may come to you, the consciousness of having contributed in some manner to the progress and welfare of humanity.

PREFACE

THE main object of this handbook is to give a concise account of the chief chemical antiseptics which have been found useful for surgical purposes during the present war. Some of the publications on this subject are not readily accessible to many who wish to inform themselves as to current European practice, and the requisite information has not yet, so far as we know, been collected into a form handy for reference. It appeared, therefore, that the present work might prove of use to surgeons and others in this country who are now taking up military duties connected with the care of the wounded.

The unparalleled severity and frequency of wound infections in the present war has led to considerable advances in our knowledge of antiseptics and of methods for their successful employment. These advances have already proved to be of great value in the treatment of septic conditions in civil and industrial practice.

No endeavor has been made to make a complete compendium of the innumerable antiseptics and disinfectants that have been proposed from time to time, for text-books already exist in which most of these substances are adequately described, and no good purpose would be served by duplication. Our principal aim has been to collect in convenient form the methods of preparation and use of various new antiseptics and modifications of old ones which have received some measure of endorsement by military surgeons during the past three years.

The utility of antiseptics, properly used as adjuncts to efficient surgery, is becoming more firmly established as the war proceeds. Surgeon General Sir George Makins in the Hunterian Oration for 1917 says: "The most useful practical test of the efficiency of any method of wound treatment is furnished by the observation of the dates at which micro-organisms disappear from the surface of the exposed tissues and at which the wound may be safely and permanently closed by suture or other means. When subjected to this test the antiseptic method has proved itself more rapid and more trustworthy than the phylacagogic or saline method of treatment."

A large part of the investigations on antiseptics carried on during the last three years has been done at the instance of the British Medical Research Committee. We are indebted to that Committee and to the editors of the British Medical Journal and the Journal of the Royal Army Medical Corps for permission to use parts of the reports published in the journals referred to.

Purely surgical details concerning the use of antiseptics do not lie within the scope of the present book although some of the principles essential to the successful use of antiseptics are briefly referred to. A short statement of the use of antiseptics in the treatment of carriers of infectious organisms is, however, included. Owing to similarity in the substances used we have deemed it advisable to include a brief statement of the use of certain disinfectants of the chlorine group for the sterilization of drinking water and the disinfection of hospital ships.

September 1, 1917.
THE HERTER LABORATORY
NEW YORK.

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A HANDBOOK OF ANTISEPTICS

CHAPTER I

GENERAL INTRODUCTION

THE terms "antiseptic," "disinfectant," and "germicide" are frequently used irrespective of their precise significance. Strictly speaking, an antiseptic is a substance which inhibits the reproduction of microörganisms, but it need not of necessity manifest great killing or "germicidal" action on such organisms. Substances such as boric acid or sodium benzoate are examples of compounds which are fairly effective in restraining the multiplication of bacteria though possessing feeble germicidal properties. Most of the substances used in the prevention of wound sepsis possess both antiseptic and germicidal properties though perhaps they are most commonly termed antiseptics.

The term "disinfectant" should clearly connote a substance which destroys infecting agents and hence is identical with a germicide, but the word has come to be used in a popular sense, irrespective of complete sterilization, to indicate some of the phenomena commonly associated with efficient disinfection, such as a deodorant effect.[1] The use of the words "disinfectant" and "disinfection" in any other sense than that first indicated is to be deprecated.

[1] An account of many important hygienic applications of disinfectants which are beyond the scope of the present work will be found in Colonel Lelean's book on "Sanitation in War." Churchill. London.

Classification of Antiseptics. — For various reasons it is quite impossible to formulate a perfectly logical classification of antiseptics. In the first place, almost every soluble substance, provided it can be obtained in sufficient concentration, is capable of exerting some antiseptic action, so that theoretically it would be necessary to classify most known organic and inorganic substances, and such an undertaking is obviously useless and unnecessary. For practical considerations it is sufficient to limit ourselves to those substances which have found fairly extended use as antiseptics. In classifying these we may endeavor to divide them according to the nature of the substances themselves or according to their mode of action. The latter method has much to commend it, but unfortunately we know but little of the intimate details of the act of disinfection as carried out with even the simplest substances, such as phenol or hydrogen peroxide.

Chemical antiseptics naturally fall into two main classes of compounds — inorganic substances as exemplified by bodies such as hydrogen peroxide, chlorine, and metallic salts, such as those of mercury and silver, on the one hand, and on the other organic, or carbon, compounds such as phenols, aromatic acids, dye-stuffs, such as malachite green, etc., etc. But in these two main groups of inorganic and organic antiseptics are found substances of widely different properties and above all showing enormous variations in *stability*. This instability of some of the most widely used antiseptics is a point of fundamental importance, for upon a clear recognition of this fact will depend, to a considerable extent, success or failure in their practical use. This chemical instability is of various kinds, as a few examples will serve to show.

Hydrogen peroxide is a good example of an unstable inorganic disinfectant. The exact way in which it destroys microörganisms is not clear but is undoubtedly connected with its oxidizing action. Now when hydrogen peroxide is

placed on the surface of an infected wound, it is rapidly decomposed, part of the oxygen of the peroxide being used up in oxidizing organic compounds and much of it being liberated as oxygen gas owing to the decomposition of the peroxide by a widely distributed enzyme, "catalase." The net result is that after a period of time which may be almost incredibly short no undecomposed hydrogen peroxide remains and disinfection proceeds no further towards completion. For, as will be shown later, the rate at which disinfection proceeds is directly related to the concentration of the antiseptic. Obviously, this instability indicates that hydrogen peroxide should be either frequently renewed or restricted in its use to conditions where temporary action only is required.

The chlorine group of antiseptics includes a number of important substances such as chlorine itself, hypochlorous acid and its sodium and calcium salts, and organic "chloramines" — *i.e.* substances containing chlorine attached to nitrogen in the form of NCl groups. They are all characterized by marked instability, since in disinfection they react not only with the cell constituents of microörganisms but also with most other substances which are apt to accompany bacteria. In so reacting, the active chlorine of the antiseptic is eventually converted either into inert chlorides or into inert organic substances in which the chlorine has become united to carbon. Thus in using the chlorine antiseptics, as with hydrogen peroxide, the process of disinfection will only go on so long as some of the active substance remains undecomposed, and in practical use means must be taken for the renewal of these antiseptics at suitable intervals, in cases where sterilization is not promptly completed.

In the examples of unstable antiseptics just cited, the active substances are decomposed during disinfection and *cannot be regenerated.* In another large class of antiseptics the active agent is not totally destroyed during disinfection but is rendered relatively inactive. Good examples of this

are found among many metallic salts; mercuric chloride and silver nitrate, for example, are much less effective when acting on bacteria suspended in hard water than when acting on washed bacteria suspended in distilled water. Similarly, phosphates and other salts frequently found in wound exudates inhibit the action of metallic antiseptics. In these cases disinfection by the metallic salts is impeded not by the complete destruction of the compound, as with hydrogen peroxide or chlorine antiseptics, but through the conversion of the metallic salt into an inactive form. This conversion may be effected either by precipitation of the metallic component in an insoluble form, or by changing its state — *e.g.* changing its condition of ionization in aqueous solution. In such cases the antiseptic substance is rendered inert but not destroyed and, theoretically at least, could be regenerated by suitable chemical means.

Lastly, there are other substances used as antiseptics which apparently are stable during disinfection or at least comparatively so. Familiar examples of compounds of this type are found in the phenols and in aromatic substances of the dye class. These compounds apparently are not readily decomposed by the cell constituents of bacteria nor by most substances which are apt to accompany the latter. It has been suggested that some of these antiseptics act by virtue of changes they produce in the surface layer of the bacterium so that the latter is rendered permeable by the antiseptic. But even with these relatively stable substances there is a definite limit to the amount of disinfection which any given quantity of the compound can accomplish and this amount will vary according to the conditions under which the antiseptic acts. This fact will be noted again in the following section in which some reference is made to the laws governing disinfection.

The following table shows the relative stability or instability of some of the common groups of antiseptics:

INORGANIC ANTISEPTICS

Hydrogen Peroxide and some of its derivatives	Unstable, easily decomposed during disinfection
Chlorine	Unstable, easily decomposed during disinfection
Hypochlorous Acid and its Salts	Unstable, easily decomposed during disinfection
Bromine and Iodine	Less unstable than chlorine
Boric Acid and its Salts	Stable
Mercury Salts	Often inactivated by precipitation or otherwise
Silver Salts	Often inactivated by precipitation or otherwise
Bismuth Salts	Often inactivated by precipitation or otherwise
Zinc Salts	Often inactivated by precipitation or otherwise

ORGANIC ANTISEPTICS

Alcohol, Ether, etc.	Stable
Iodoform	Fairly stable
Formaldehyde	Unstable
Hexamethylenetetramine and its derivatives	Mostly stable
Phenols, Naphthols, and derivatives	Mostly stable
Aromatic Chloramines	Unstable, easily decomposed during disinfection
Dyes such as malachite green, acriflavine, etc.	Mostly stable, though sometimes reduced to leuco-forms, and often adsorbed by tissues

It is a surprising fact that this varying stability of antiseptics is so little realized by many workers. Current literature is full of reports of experiments in which bacteriologists have added reactive unstable substances, such as hypochlorous acid, to media which cause their prompt disappearance in dilute solution; the mixture containing little or no remaining antiseptic is subsequently sown with organisms and astonishment is expressed at the inefficiency of the substance employed as a disinfectant. This important point will be referred to repeatedly in later sections.

THE LAWS GOVERNING DISINFECTION. — These have been chiefly worked at by Miss Chick.[1] Earlier experiments were

[1] Journal of Hygiene, *8*, p. 92, 1908, and *10*, p. 238, 1910.

carried out by Krönig and Paul[1] and by Madsen and Nyman.[2] It would be out of place to go into the details of the elaborate experiments and calculations necessary to elucidate the principles of disinfection but a brief summary of the essential results may be given. Miss Chick observed the number of bacteria that survived, at varying intervals of time, the action of a constant quantity of a stable antiseptic, such as phenol, at a constant temperature, on a known number of organisms. By utilizing these results mathematically she was able to calculate a velocity coefficient for the disinfecting action of the substance. The main result of these experiments was to show that in all essential particulars the act of disinfection could be regarded as obeying the laws governing a simple chemical reaction, the disinfectant representing one reagent and the bacteria the other. This conception is of the greatest importance since the cardinal points of efficient disinfection, namely adequate active mass or concentration of antiseptic, time of action, and perfect contact, are thereby experimentally established.

The influence of temperature upon the rate of disinfection is interesting since here again a close analogy exists between ordinary chemical reactions and disinfection. The velocity of disinfection increases with rise in temperature in a manner similar to that of an ordinary chemical reaction. Some idea of the magnitude of the effect of temperature may be gathered from the fact that with metallic salts the mean velocity of disinfection increased 2–4 fold for a rise in temperature of 10° C., while with phenol it was as high as eightfold, using *B. paratyphosus* as test organism in each case.

It must be remembered, however, that the similarity in the mathematical relations governing the velocity of simple chemical reactions and disinfection does not of necessity prove that the act of disinfection is a chemical one, although with some antiseptics this is almost certainly the case.

[1] Zeitschr. f. Hygiene, *25*, p. 1, 1897. [2] *Ibid.*, *57*, p. 388, 1907.

INFLUENCE OF MEDIA. — The capacity of a disinfectant to kill microörganisms is dependent to an extraordinary degree upon the conditions under which it acts. Almost invariably the greatest germicidal activity is shown when the substance acts upon bacteria freed from contaminating culture media and suspended in distilled water or salt solution. The presence of proteins and similar substances, *e.g.* peptones, usually causes a huge reduction in the germicidal activity of most substances. In the presence of pus, in which many of the organisms are partly ingested in the bodies of the dead leucocytes and hence difficult to attack, the results are still less favorable. The following table gives some idea of the reduction in germicidal activity of some of the common antiseptics acting for two hours at room temperature on *staphylococcus aureus* (1 drop of 24 hours broth culture) in a total volume of 5 cc. of either water or 50 per cent horse serum. The antiseptics were in all cases added last of all to the mixtures:

ANTISEPTIC	STAPHYLOCOCCI IN WATER	STAPHYLOCOCCI IN 50% HORSE SERUM
Phenol	1 : 250 −	1 : 50 −
	1 : 500 +	1 : 100 +
Salicylic Acid	1 : 2500 −	1 : 100 −
	1 : 5000 +	1 : 250 +
Hydrogen Peroxide	1 : 3500 −	1 : 1700 −
	1 : 8000 +	1 : 2000 +
Iodine	1 : 100,000 −	1 : 1000 −
	1 : 1,000,000 +	1 : 2500 +
Mercuric Chloride[1]	1 : 5,000,000 −	1 : 25,000 −
	1 : 10,000,000 +	1 : 50,000 +
Silver Nitrate[1]	1 : 1,000,000 −	1 : 10,000 −
	1 : 10,000,000 +	1 : 25,000 +
Sodium Hypochlorite	1 : 500,000 −	1 : 1500 −
	1 : 1,000,000 +	1 : 2000 +
Chloramine-T	1 : 500,000 −	1 : 2000 −
	1 : 1,000,000 +	1 : 3000 +

The − sign indicates sterilization as evidenced by negative subcultures while the + sign indicates incomplete disinfection.

[1] No antidote was used in these experiments to prevent the action of traces of the salt in the subcultures. (Cp. p. 52.)

In order to illustrate the difficulty of sterilizing pus, some selected experiments made by W. Parry Morgan [1] may be cited. In these experiments one part of pus was mixed with nine parts of antiseptic and after ten minutes 10 cmm. were planted in liquid agar which was then shaken and incubated. It was found that when the antiseptic was strong the number of colonies could be counted readily, but when it was weak the agar became opaque with innumerable colonies. The results are, of course, comparative and do not lend themselves to strict quantitative expression, for the specimens of pus vary much among themselves.

TABLE SHOWING THE EFFECTS OF ANTISEPTICS UPON THE GROWTH OF BACTERIA IN PUS

Iodine	1:400	1:800	1:1600	1:3200
16 hours	+	++	+++	+++
Mercury Biniodide	1:400	1:800	1:1600	1:3200
16 hours	+	++	++	+++
Phenol	1:40	1:80	1:160	
16 hours	+	++	+++	
Sodium Hypochlorite (Dakin's Solution)	1:200	1:400	1:800	1:1600
18 hours	−	−	−	+
3 days	−	−	−	+
Eusol (Bleaching Powder and Boric Acid) available chlorine	1:200	1:400	1:800	1:1600
18 hours	−	−	−	+
3 days	−	−	−	++

When the proportion of pus to antiseptic was higher than in the above experiments, the disinfecting action was still further diminished.

A few substances, such as some derivatives of hexamethylenetetramine, are known whose germicidal action is said not to be materially affected by the presence of serum, and one striking example exists of a substance which is apparently actually more active in serum than in water. This compound prepared by Benda and termed trypaflavine but now known

[1] Brit. Med. Journ., May 13, 1916.

as acriflavine (sometimes simply flavine) has been shown by Browning [1] and his colleagues to kill staphylococci in serum at one tenth the concentration necessary when the same organisms are suspended in peptone solution. Experiments with pus, however, showed much higher concentrations were necessary to achieve sterilization than with peptone or serum media.

The causes of these huge variations in the germicidal action of antiseptics under various conditions are but slightly understood. The reduction in the case of members of the chlorine group can be accounted for to a considerable extent by the rapidity of the chemical interaction of the disinfectant with the protein medium, with the production of inert substances if the proteins be in sufficient excess. But other causes must be at work where the more stable antiseptics such as phenol and the dye-stuffs are concerned. It is frequently said that the antiseptic is "quenched" or "fixed" by the protein medium, but these terms do not carry much enlightenment, nor does the suggestion that the molecular condition of the antiseptic is influenced by the presence of colloids in the medium. The low germicidal action shown by most antiseptics against pus is due in part no doubt to the mechanical difficulties of penetrating the mucoid particles in the pus. Parry Morgan has shown, moreover, that when the organisms in pus, or added to it, have undergone phagocytosis they are less readily destroyed by antiseptics. The reduction in antiseptic activity of a substance in the presence of any particulate matter has been often observed [2] and is probably connected with the surface adsorption of the antiseptic by the particles with consequent reduction in its effective concentration.

The Choice of Antiseptics. — The selection of antiseptics for various purposes requires the consideration of a

[1] Brit. Med. Journ., Jan. 20, 1917.
[2] Chick and Martin, Journ. of Hygiene, *8*, p. 654, 1908.

number of factors. The possession of high germicidal activity, as ordinarily tested in the presence of media comparable with those in which the disinfectant is to act, is of course desirable. But it is equally important to bear in mind the concentration at which the substance may be used, for a relatively innocuous substance used in fairly high concentration will often give much more satisfactory results than lower concentrations of more active substances. The speed of disinfection is also an important question for it varies enormously with different types of antiseptics. Antiseptics of the chlorine group and iodine are among the most rapid, while the dyes and some metallic salts are relatively slow. An inspection of the tables in Chapter VII, in which a heavily infected mixture of blood serum and muscle extract was treated with various antiseptics at about the concentration recommended for wound treatment will give a good idea of their relative speed of action and potency under the conditions selected.

The ideal surgical antiseptic should effect complete sterilization within its sphere of action without causing any damage to animal cells. At the moment such a substance does not appear likely to be found, but on the other hand it is surprising to see how little damage may be done to animal tissues by some active antiseptics. An important method of judging of the injurious action of antiseptics is to investigate the condition of the leucocytes in wounds recently treated with the substance under consideration. In general it appears from experiments *in vitro* that, with the strength of antiseptics commonly used in surgery, mercury salts and hypochlorites have relatively little effect on phagocytosis as compared with phenol (Parry Morgan). It is a regular phenomenon to observe activity of the leucocytes obtained from wounds which have been recently treated with hypochlorites.

Ingenious methods for determining the influence *in vivo*

of antiseptics on the activities of leucocytes have been worked out by Col. C. J. Bond.[1] Indigo impregnated threads are laid in aseptic or septic wounds with or without antiseptics. After varying periods of time the threads will be found to have become decolorized owing to the ingestion of pigment particles by the leucocytes. It was found that the application of a strong antiseptic, *e.g.* 1 : 1000 mercury biniodide or 1 : 20 phenol, to a wound such as that made for the radical cure of hernia or for the removal of varicose veins, does delay to some slight extent the decolorization of an indigo thread placed in it as a drain. But in general it appeared that antiseptic solutions in moderate concentration exercise less influence over emigration and phagocytosis than many surgeons had supposed. There are, however, reasons for concluding that many antiseptics do exert a considerable inhibitive effect on the return immigration of living phagocytes. But if the use of antiseptics does bring about a reduction of the numbers, or inhibit the activities, of pathogenic organisms, not merely *in vitro* but in the actual wound, then although these reagents undoubtedly do cause the death of a certain number of body cells and prevent others from again reaching the tissues, this is relatively of slight importance if the invading organisms are at the same time materially diminished in numbers or offensive capacity.

Other criteria which need consideration in judging of the suitability of any particular substance as an antiseptic concern the absence of marked irritation of the skin or other tissues to which the substance may be applied, and also its effect on the rate of dissolution of necrotic tissue and the rate of formation of healthy granulations. In general, too, it will be found that antiseptics which coagulate the proteins in wound exudates, *e.g.* salicylic acid, or strong phenol, are less desirable than those which do not act in this way,

[1] Brit. Med. Journ., June 3, 1916, Feb. 3, 1917.

since anaërobes are apt to multiply in necrotic tissue more or less surrounded with impermeable coagulum. In contrast with this effect, the solvent action of hypochlorites and the related dichloramine-T on dead tissue is marked and constitutes a valuable property.

But the problem of selecting the most desirable antiseptic is by no means limited to the preceding considerations. It is of paramount importance that judgment should be exercised in choosing a substance which is likely to be effective under the conditions of its employment. Thus any of the hypochlorite solutions which give excellent results when used for the intermittent flushing of infected wounds, partly on account of the rapidity of their action and of their generally inoffensive character, are almost useless when applied in small amounts as a simple wet dressing which is infrequently renewed. In the latter case no active antiseptic persists for any significant length of time and not much more is accomplished than the prevention of secondary reinfection. When prolonged action is required and frequent application is impossible, recourse must be had to more stable mixtures which yield up their store of antiseptic slowly. Examples of such substances are the bismuth iodoform paraffin mixture (B.I.P.) and the oily solution of dichloramine-T. These substances will be referred to later.

Finally, it must be once more emphasized that antiseptics at best are only accessories to, and not substitutes for, efficient surgical treatment of infected wounds. The disinfecting action of antiseptics is practically restricted to the wound surfaces and the cavity inclosed by them and none of them appears to have any considerable power of penetration without simultaneous loss of activity. The prompt excision of damaged tissue has now become a routine method in war surgery on the Western Front, and this procedure renders the effective employment of antiseptics much more certain than it was earlier in the war.

MODES OF APPLICATION. — Antiseptics are applied in various ways, according to the character of the wound, the nature of the substance, and the kind of action desired. Aqueous solutions are used most extensively. When unstable antiseptics, such as hypochlorites and chloramine-T, are used and prolonged antiseptic action is required — as in the treatment of freshly infected or septic wounds — it is necessary to employ some means of frequently renewing the antiseptic. This end is commonly accomplished either by irrigation or by intermittent instillation of fresh antiseptic solution into the wound by means of short rubber tubes so arranged that the solution may reach every recess of the wound. This method has been used extensively in the present war and has given excellent results. The technique of the method will be found fully described in a book by Carrel and Dehelly.[1] To obtain the best results with this mode of treatment, a careful observation of details is essential, and as only an outline can be given here reference should be made to the book just mentioned or to numerous original papers in recent publications.

The first step is the mechanical cleansing of the wound by excision, this being omitted as dangerous if the phenomena of inflammation have already set in. The wound is then subjected to active antiseptic treatment. For this purpose sodium hypochlorite — so-called Dakin's solution — is employed at a concentration of 0.5 per cent (see page 23). Great care is taken to insure uniform distribution of the antiseptic by means of the rubber tubes which are usually of fine bore, closed at one end and provided near the closed end with a number of fine lateral perforations. A number of these tubes, depending on the size of the cavity, are disposed within the wound, care being taken that no gauze intervenes between the tubes and the

[1] Le traitement des plaies infectés, Collection Horizon, Masson et Cie., Paris, 1917.

tissues and also that the tubes are not so closely applied as to block the perforations. The tubes are led through the outer dressing and kept in position with safety pins. When, as is always the case with large wounds, more antiseptic solution is needed than can be conveniently supplied by using a glass syringe, the ends of the tubes are connected by T pieces to a single tube which in turn connects with a glass reservoir holding a liter of solution which is suspended several feet above the level of the bed. The supply of solution to the wound is arranged by opening for a few seconds every two hours the clip attached to the main tube. An important point is the absence of a drainage opening at the most dependent part of the wound; in fact, the ideal arrangement is attained when the wound is cup-shaped; when it is on the inferior surface of a limb the aperture is partly plugged with gauze to hinder free escape of the fluid. It is important to note that vessels in the base of the wound should not be ligatured with silk since this material is attacked by hypochlorites. The routine bacteriological examination of the wound is important. It is found that when practical sterility has been maintained for two or three days it is safe to close the wound. In the case of wounds that have suppurated, it is advisable to wait a little longer.

In place of the hypochlorite, the more stable chloramine-T has been used to a considerable extent and with particular success in fresh industrial accidents. It is usually used in 0.5–2 per cent solution and its action is similar to that of hypochlorite save that necrotic tissue is less rapidly removed.

The methods of application of the ordinary solutions of less unstable antiseptics such as phenol, iodine, and the metallic salts, call for no special comment.

When the frequent renewal of the antiseptic is impracticable or not desired, recourse may be had to pastes or oils of various kinds, which embody a store of antiseptic that may be gradually utilized. The mild antiseptic action

of a paste containing about one per cent of chloramine-T and five per cent of sodium stearate in water is often useful to maintain sterility and prevent reinfection of wounds which have previously received more vigorous treatment. A much more intensive action can be obtained from the use of dichloramine-T dissolved in eucalyptol and paraffin as described on page 39. The amount of active antiseptic which may thus be applied to the wound surface is extremely large and its action is correspondingly prolonged. The method of using this oil is particularly simple and the results appear to be exceptionally good, both as regards the prevention and treatment of sepsis.

A paste possessing moderate potency, introduced by Rutherford Morison, composed of bismuth subnitrate, iodoform, and paraffin, is being widely used with good results both for wounds of the soft tissues and for fractures. The fact that the wounds do not need dressing for several days gives it a great advantage when the number of cases requiring treatment is very large, and because of this it also secures a maximum of rest for the wound.

It must not be forgotten that the employment of pastes as a primary dressing is not free from serious risks and should only be practiced with caution. The use of salicylic acid pastes for the immediate treatment of wounds other than superficial ones is said to have given particularly poor results as anaërobes are apt to flourish in walled off recesses of the wound. The coagulating properties of salicylic acid would especially facilitate such a result.

The administration of antiseptics by intravenous injection is the only method other than that of local application that is at all practiced for military purposes. Eusol has been used to some extent for this purpose [1] though it is abundantly clear that any action it may have is unconnected with any

[1] Lorrain Smith, Ritchie, and Rettie, Brit. Med. Journ., Nov. 13, 1915. Frazer and Bates, Brit. Med. Journ., Aug. 5, 1916.

germicidal effect since the actively hæmolytic hypochlorite, in therapeutic doses, is immediately decomposed by the blood.[1] A few striking cases of favorable results of its use have been recorded but as the procedure has not found wide acceptance and is not free from danger, it is well to suspend judgment as to its value. A number of years ago[2] silver nitrate was used intravenously in the same way and while it was clear that no direct germicidal effect was possible, some kind of beneficial effect was believed to follow the hæmolysis caused by the silver salt.

[1] Dakin, Brit. Med. Journ., June 17, 1916.
[2] Klinisch-Therapeutische Wochenschrift, No. 33, p. 881, 1908.

CHAPTER II

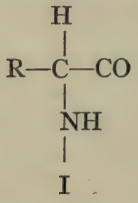
ANTISEPTICS OF THE CHLORINE GROUP

In the present war, which is distinguished by the frequency and intensity of virulent wound infections, no class of antiseptics has received such extensive employment as those of the chlorine group. When properly applied — and this is an important reservation — it is generally conceded that they have proved of genuine value to the surgeon. All of the antiseptics of this group are characterized by chemical instability in the presence of organic matter, and therefore conditions favorable for their use must include either provision for their frequent renewal, or the use of some immiscible solvent for the antiseptic so that the active compound may be gradually liberated.

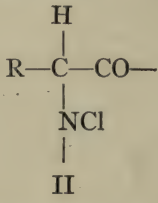
The members of the group under consideration are often spoken of as containing "active" chlorine as distinct from inert chlorine such as that in common salt. It must not be inferred, however, that the phrase "active chlorine" necessarily implies either that free chlorine is contained in the substance or is liberated from it, as more often it will be found that hypochlorous acid or some such compound is the active agent. From the standpoint of disinfection, it is probably correct to connote with the term "active chlorine" in a compound, the ability of any particular substance to part with chlorine, free or combined, in such a way that it can effect the chlorination of bacterial and other proteins.[1]

[1] All compounds containing active chlorine possess the property of liberating iodine from an acidified solution of potassium iodide. The iodine may be detected by the development of a blue color on adding starch paste. This reaction will often be found useful in testing for the presence of unchanged antiseptic.

This chlorination of bacterial protein seems to be incompatible with the life of the microörganism. It is necessary to refer in outline to the nature of this reaction. All proteins, irrespective of their origin, contain large numbers of amino-acid groups, which may be represented as shown below: (I)



I



II

These groups are capable of attack by substances containing active chlorine in such a way that the hydrogen attached to the nitrogen atom is replaced by chlorine (II). The compounds thus formed contain the (NCl) group and hence belong to the class of chloramines. Their chlorine is still active and they are themselves active germicides. Other concomitant reactions also occur which use up part of the chlorine, converting it into an inert form, *e.g.* the chlorine becomes united to carbon or forms chlorides.

This formation of germicidally active chloramines is of importance in several respects. For example, the proteins and other nitrogenous compounds present in wound secretions may be converted into chlorine derivatives of antiseptic value by the action of a sufficient quantity of hypochlorite or similar substance.[1] While preformed chlorine derivatives

[1] It is of interest to note that while chlorine, bromine, and iodine have not widely differing germicidal properties, hypobromites and hypoiodites in contrast to hypochlorites have but trivial disinfecting action. Correlated with this fact is the observation that hypochlorites react readily with proteins while hypobromites and hypoiodites do not. On the other hand, certain synthetic bromamines which react readily with amino-acids and proteins have a high germicidal potency. The ability to react with proteins and allied bodies is clearly associated with germicidal activity in members of the halogen group of antiseptics.

prepared from proteins are powerful germicides, they are not convenient substances for general use, but by using other types of nitrogen compounds for chlorinating, synthetic chloramines, with valuable antiseptic properties, are readily obtainable.

For detailed information concerning the action of chlorine antiseptics upon amino-acids, proteins, etc., as well as the preparation of many synthetic chloramines, reference must be made to the original papers.[1]

In addition to their disinfecting action, the chlorine antiseptics are strong oxidizing agents and deodorants and moreover possess in high degree the property of decomposing toxins. By the regulated action of hypochlorous acid, Dean [2] has prepared a non-toxic dysentery vaccine and it is a common observation that the free use of hypochlorites may reduce the constitutional symptoms arising from septic processes and that they reappear on discontinuing the antiseptic treatment.

The antiseptics of the chlorine group which are most commonly employed in the treatment of infected wounds, are the following: [3]

(*a*) Hypochlorous acid and its sodium and other salts (including "eupad," "eusol" and so-called Dakin's solution).

(*b*) Chloramine-T, the abbreviated name for sodium toluene sulphonchloramide.

(*c*) Dichloramine-T, the abbreviated name for toluene sulphondichloramine.

In most respects, the action of these various chlorine compounds is essentially similar, though each possesses certain properties which render it more or less suitable for

[1] Brit. Med. Journ., Jan. 29, 1916, June 17, 1916; Proc. Roy. Soc. B, *89*, p. 232, 1916. Biochem. Journ., June, 1917.

[2] Brit. Med. Journ., April 29, 1916.

[3] Chlorine water has been used to some extent, but it is doubtful whether its use is preferable to that of the more convenient iodine solution, which in most respects it resembles.

particular purposes. As a matter of convenience, it may be desirable to give a short résumé of these considerations.

I. *Hypochlorous acid and hypochlorites* are best suited to cleansing septic wounds by irrigation. They markedly assist in the dissolution of necrosed tissue. They are unstable and very reactive, and must be frequently renewed to all parts of a wound, this being best achieved by the method of intermittent instillation (p. 13). They are the cheapest antiseptics of the chlorine group and are much cheaper than other effective germicidal substances. Free hypochlorous acid is more irritating than the sodium salt. The latter in 0.5 per cent neutral or feebly acid (boric acid) solution may be used in large quantities under appropriate precautions for a considerable time without causing irritation. The skin is more susceptible than the deeper tissues and should be protected with vaseline or some similar substance.

II. *Chloramine-T* can be used in stronger solution (up to two per cent) than the hypochlorites. It is more stable and exerts more prolonged antiseptic action and is considerably more effective than hypochlorite when acting in the presence of much blood. It is not toxic and is less irritating than the hypochlorites and has but little solvent action on necrosed tissue. It is well suited for use on wounds previously cleansed with hypochlorites or dichloramine-T, and in suitably dilute solutions may be used in the eye and on other sensitive parts. It may be applied in solution, as an impregnation of gauze, or in a sodium stearate cream.

III. *Dichloramine-T* dissolved in oily media may be sprayed upon wound-surfaces or poured into accessible parts of deep wounds. It yields moderate amounts of antiseptic to watery media such as secretions from wounds or mucous membranes. It is suitable for cases requiring prolonged antiseptic treatment, and for first dressings of recent wounds which do not require irrigation. It is also used for nasal antisepsis (p. 102). Dichloramine-T in oil solution has a

great advantage over the other chlorine antiseptics in that it may be used in high concentration, and its action is of much longer duration. The application of the oil is extremely simple and it ordinarily need not be renewed more than once in 24 hours.

Hypochlorous Acid and its Salts

The disinfecting action of these substances has been known for over a hundred years and they have received numerous hygienic applications. An account of their early history and uses will be found in the British Medical Journal, Dec. 4, 1915. In the early part of the present war, several surgeons made use of commercial sodium hypochlorite, "eau de Javel," for the treatment of infected wounds, but there were many accidents owing to its caustic action, which was largely due to the presence of excessive amounts of alkali in the solution. Apart from this objectionable property, the substance appeared to possess desirable qualities and it was for this reason that several workers sought to obtain hypochlorite solutions which were less irritating but which retained their germicidal properties unchanged.

These various solutions are all prepared from bleaching powder (chloride of lime) which is the most readily accessible solid source of hypochlorous acid and its salts. Bleaching powder is prepared by the action of chlorine upon slaked lime and in most respects behaves like a mixture of calcium hypochlorite and calcium chloride. It is of variable composition and slowly decomposes on keeping, especially when exposed to air or light (p. 41). In making some of the antiseptic solutions, the calcium hypochlorite of the bleaching powder is converted into sodium hypochlorite by the action of sodium carbonate. In most of them boric acid is added to counteract the objectionable alkalinity of ordinary hypochlorites, and this has also the effect of liberating a certain

amount of free hypochlorous acid, leaving the solution neutral or faintly acid. Sodium hypochlorite may also be prepared by the electrolysis of sodium chloride solution (p. 116) and by the action of chlorine on sodium carbonate or sodium bicarbonate. It will be impossible to describe the preparation and uses of all the various hypochlorite solutions that have been employed for surgical use and it would appear sufficient to give an account of the two that seem to be most widely used, namely "eusol" and the so-called Dakin solution. All hypochlorite solutions attack metals and hence they should not be used for the sterilization of instruments.

EUPAD AND EUSOL.[1] — These are preparations of bleaching powder and boric acid, either dry (eupad) or in solution (eusol). The former is prepared by intimately mixing equal weights of bleaching powder and boric acid, both in fine powder. It has been employed as a dusting powder, and in strands of gauze for drainage or between layers of moistened gauze or lint as a dressing. When moistened, eupad liberates hypochlorous acid partly in gaseous form and in variable amount and this, if excessive, is liable to prove highly irritating, so that the quantity of eupad used must be carefully controlled. Eupad forms a rather thick, white coagulum with wound exudates and this may occasion inconvenience. It is used much less extensively than is the aqueous eusol. An almost identical mixture was recommended by Vincent some years ago while Lumière advocates a mixture containing bleaching powder one part with boric acid three parts.

Eusol is prepared in either of two ways:

(1) Twenty-five grams of eupad are shaken up with one liter of water, allowed to stand for a few hours, then filtered through cloth or filter paper.

[1] Lorrain Smith, Drennan, Rettie, and Campbell, Brit. Med. Journ., July 24, 1915.

(2) To 1 liter of water add 12.5 grams bleaching powder, shake vigorously. Then add 12.5 grams boric acid powder and shake again. Allow to stand for some hours, preferably overnight, then filter off, and the clear solution is ready for use. Eusol prepared in this way from good quality bleaching powder contains the equivalent of about 0.27 per cent hypochlorous acid.[1] The solution gives thick precipitates containing calcium with blood or wound exudates and is strongly hæmolytic. It is frequently spoken of as a solution of hypochlorous acid but actually the mixture is alkaline to litmus and contains a balanced mixture of calcium hypochlorite and borate with an undetermined amount of free hypochlorous acid. The separate estimation of the latter is a difficult problem. A large number of experiments on the germicidal action of eusol will be found in Lorrain Smith's paper, together with suggestions as to methods of use in the treatment of infected wounds. It is recommended by its authors for use (*a*) as a lotion, diluted if necessary, (*b*) as a fomentation, (*c*) as a wet dressing with gauze without a waterproof covering, and (*d*) as a bath, diluted if necessary.

The general principles concerning the use of chlorine antiseptics as germicides apply equally to eusol and the other hypochlorites (cp. pp. 12, 20).

Neutral Sodium Hypochlorite Solution ("Dakin's Solution"). — This preparation is essentially a solution of sodium hypochlorite, containing 0.45 to 0.5 per cent NaClO made in such a way that it is, and remains, substantially neutral. Ordinarily commercial hypochlorite is very variable in composition and commonly contains much free alkali and occasionally free chlorine. Such solutions are very irritating and should not be used for surgical purposes. The original formula[2] for making the neutral solution requires

[1] Through an error in calculation, the composition was first given as 0.54 per cent. The other analytical figures given in the original paper also contain errors.

[2] Comptes rendus, *161*, p. 150, 1915, Brit. Med. Journ., Aug. 28, 1915.

the use of boric acid for neutralization. The reason for this may perhaps be briefly referred to. It is well known that blood and some other body fluids and also certain artificial salt solutions containing mixtures of the salts of polybasic acids — *e.g.* phosphoric or carbonic acid — are able to retain their essential neutrality even after the addition of limited quantities of acid or alkali. This is due to the fact that the addition of acid or alkali simply changes the relative proportion of two or more salts of the polybasic acid present in the solution. Such solutions are often referred to as "balanced" and the salts in them are called "buffer salts." Utilizing the same principle and employing the feeble polybasic boric acid, a simple balanced hypochlorite mixture was prepared which maintains essential neutrality under all conditions. It should be understood that the insignificant antiseptic action of boric acid has nothing to do with the employment of this acid nor is the boric acid employed for the purpose of liberating free hypochlorous acid as in Lumière's or Lorrain Smith's preparations.

Preparation of Neutral Sodium Hypochlorite. — One hundred and forty grams of *dry* sodium carbonate (Na_2CO_3) or 400 grams of the crystallized salt (washing soda) are dissolved in 10 liters of tap water, and 200 grams of bleaching powder containing 24–28 per cent of "available chlorine" are added.[1] The mixture is very thoroughly shaken, both to make good contact and to render the precipitated calcium

[1] Bleaching powder varies considerably in its available chlorine content, though when bought in bulk the fresh product is fairly constant in composition. It is advisable to determine the "available chlorine" in each large batch of bleaching powder purchased, as described on p. 41. Bleaching powder with less than 23 per cent of available chlorine should be rejected. Exceptional samples may contain as high as 35 per cent available chlorine and in such cases it is well to reduce correspondingly the ingredients taken in the above formula. For purposes of rough calculation, one may assume that using 200 grams of bleaching powder for 10 liters of solution, the resulting product will contain as much sodium hypochlorite as is represented by the available chlorine of the bleaching powder divided by 50. Thus 25 per cent "available chlorine" bleaching powder will give 0.5 per cent sodium hypochlorite solution.

carbonate granular and promote its settling. It is then allowed to stand quietly and after half an hour the clear liquid is siphoned off from the precipitate and filtered through a cotton plug or paper. Forty grams of boric acid are added to the clear filtrate and the resulting solution is ready for use. The boric acid must not be added before filtering but only afterwards. The exact strength should be determined from time to time, as directed on p. 41. It is important that the solution should not be stronger than 0.5 per cent sodium hypochlorite or irritation of the skin may be frequent. On the other hand, it should not be less than 0.4 per cent or its germicidal action is materially diminished. The solution should also be tested for neutrality by adding a little of it to a trace of solid phenolphthalein suspended in water. No red color indicating free alkali should develop or else more boric acid must be added; this is, however, rarely necessary with the above proportions. The solution should not be kept longer than one week.[1]

Daufresne has shown that it is possible to prepare a satisfactory solution of sodium hypochlorite without employing boric acid for neutralization if sodium bicarbonate be used in conjunction with sodium carbonate for decomposing the bleaching powder. The relative proportions of sodium carbonate and bicarbonate required to furnish a neutral

[1] A stronger solution may be prepared by decomposing bleaching powder with dry sodium carbonate in the proportion of 150 gms. to 105 gms., dissolved in 1 liter of water. The mixture is filtered and a measured portion of it (20 cc.) rapidly titrated with a boric acid solution of known strength (31 gms. per liter, ½ normal), using phenolphthalein suspended in water as indicator (the usual alcoholic solution of phenolphthalein will not serve, because the alcohol is at once attacked) in order to determine the amount of boric acid to be added to the rest of the filtrate. (Each cubic centimeter of N/2 boric acid calls for 3 gms. boric acid to be added.) An excess of boric acid should be avoided as it favors the liberation of hypochlorous acid and renders the solution less stable. It is best to add slightly less than the calculated amount. The concentrated solution thus prepared contains about 4 per cent of sodium hypochlorite and should be mixed with 7 parts of water before use. It can be kept for a month without serious decomposition.

solution depends upon the varying composition of the bleaching powder used and cannot simply be deduced from its "available chlorine" content, as is frequently stated. The proportion of free lime in the bleaching powder is obviously as important as its chlorine strength. With some brands of bleaching powder retailed in the United States, the following proportions have proved useful:

Two hundred grams of bleaching powder (24–28 per cent available chlorine) [1] is shaken well with five liters of water and allowed to stand for an hour or two. In a separate vessel dry sodium carbonate (94 grams) and sodium bicarbonate (86 grams) are mixed with five liters of cold water, and when dissolved, the solution is added to the bleaching powder suspension and the mixture well shaken. The precipitate of calcium carbonate is allowed to settle and the clear supernatant solution is syphoned off and filtered. The solution should contain close to 0.5 per cent sodium hypochlorite and this should be checked by analysis (p. 41). If too strong it should be diluted with water to 0.5 per cent strength.

It is most important to test the solution for free alkali by adding a trace of solid phenolphthalein to a little of it. In case a red color develops indicating free alkali, the solution may still be used if it is previously neutralized either by passing carbon dioxide through the solution or by adding a little boric acid, until the alkaline reaction is abolished. But in making further quantities of the solution, using the same sample of bleaching powder, alkalinity may be avoided by reducing the quantity of sodium carbonate and correspondingly increasing the bicarbonate.

Sodium hypochlorite, whether prepared according to the preceding formulæ or according to other methods that will occur to the chemist, *e.g.* from salt by electrolysis (p. 116) or from liquefied chlorine gas, when used in neutral solution

[1] Or an equivalent amount of stronger bleaching powder.

at 0.5 per cent concentration is found to be a valuable antiseptic for the treatment of infected wounds. Its action is extremely rapid and then ceases as soon as all the hypochlorite is decomposed, hence the methods for using the solution efficiently must provide for its frequent renewal. A short account of the technique advocated by Carrel and Dehelly will be found on p. 13. For further details, reference may be made to their book and to the papers noted below.[1]

The hypochlorite solutions possess the valuable property of assisting in the rapid dissolution of necrotic tissue, doubtless owing to their ability to react with proteins with the formation of soluble products. They possess a slight but definite hæmostatic action but are actively hæmolytic and should not be injected intravenously. The hypochlorites are extremely reactive substances chemically, and should neither be heated above 37° C. or used with other antiseptics nor with alcohol nor ether.

It is rather difficult to give useful figures for the germicidal effects of sodium hypochlorite since so much depends on the capacity of the medium to decompose the hypochlorite before it can complete disinfection. Most pyogenic organisms suspended in water are killed at a concentration of less than 1 : 100,000, while in serum about 1 : 1500 is necessary. Blood decomposes the hypochlorites rapidly, so that 1 : 300 or more may be necessary before sterilization is complete. The action of sodium hypochlorite and eusol on a mixture of pyogenic and other organisms in a blood serum muscle extract mixture is recorded on p. 85. The lethal concentration under the stated conditions is probably a little less than 1 : 1000.

[1] Le traitement des plaies infectés. A. Carrel & G. Dehelly. Masson et Cie., Paris, 1917.

Carrel, Dakin, Daufresne, Dehelly, and Dumas. Presse Medicale, Oct. 11, 1915.

Tuffier, Bull. de l'Acad. de Méd., *74*, No. 38, 1915.

Dépage, A., Bull. et Mém. Soc. de chir. de Paris, *42*, p. 1987, 1916.

Lyle, H. H. M., Journ. Am. Med. Assoc., Jan. 13, 1917.

The extraordinary rapidity of its action in concentrations even lower than those employed for surgical purposes is well illustrated.

Chloramine-T

Chloramine-T is the abbreviated name for sodium toluene-sulphonchloramide.[1] It is a crystalline, odorless substance containing 12.6 per cent of chlorine. It is readily soluble in water and the solutions, which have a bitter taste, are stable, neither moderate exposure to heat nor light causing appreciable decomposition. In equimolecular solutions its germicidal activity is about four times that of sodium hypochlorite. This may, perhaps, be explained by the fact that the chlorine is already linked to nitrogen and is less rapidly appropriated through reactions with proteins and other substances in the wound secretions. The relatively slight solvent action of chloramine-T on necrosed tissue supports this view. Its antiseptic efficiency is prolonged by this reduction in reactivity. Its germicidal action is rapidly exerted and in most respects it resembles the hypochlorites closely, though decidedly less irritating than the latter. An idea of its potency may be gathered from the accompanying tables, although in some respects a better indication is given by the results recorded in a later chapter (p. 86).

In another series of experiments note was taken of the speed of disinfection. Horse blood serum (2 cc.) or 0.7 per cent Witte's peptone (2 cc.) was inoculated with *staphylococcus aureus* and subsequently treated with weak chloramine-T solutions (1 cc.). The results show clearly that the rate of disinfection is very rapid, the maximum effect being observed in a few minutes. In those cases where disinfection was incomplete and no active antiseptic persisted in the mixture, subsequent growth took place (p. 30).

[1] Dakin, Cohen, and Kenyon, Brit. Med. Journ., Jan. 29, 1916.

TABLE SHOWING THE GERMICIDAL ACTION OF CHLORAMINE-T ON SEVERAL COMMON ORGANISMS

Two drops of a fresh culture of the organisms were suspended in 5 cc. of fluid, either water or 50 per cent horse serum, and the antiseptic was allowed to act two hours at room temperature. The mixtures were then subcultured. In comparison, a few figures for sodium hypochlorite and phenol are added.

	CHLORAMINE-T.	SODIUM HYPOCHLORITE	PHENOL
Staphylococci in water . .	1 : 500,000 −	1 : 500,000 −	1 : 250 −
	1 : 1,000,000 +	1 : 1,000,000 +	1 : 500 +
Staphylococci in serum .	1 : 1,500 −	1 : 1,500 −	1 : 50 −
	1 : 2,500 +	1 : 2,000 +	1 : 100 +
B. pyocyaneus in water .	1 : 200,000 −	1 : 100,000 −	1 : 200 −
	1 : 400,000 +	1 : 1,000,000 +	1 : 400 +
B. pyocyaneus in serum .	1 : 1,250 −	1 : 2,500 −	1 : 25 −
	1 : 2,000 +	1 : 5,000 +	1 : 50 +
Streptococci in water . .	1 : 1,000,000 −		
Streptococci in serum . .	1 : 2,500 −		
	1 : 5,000 +		
B. capsulatus in water . .	1 : 1,000,000 −		
B. capsulatus in serum .	1 : 2,500 −		
	1 : 5,000 +		

Complete sterilization is indicated by −, while + indicates that organisms survived.

Chloramine-T may be used for wound treatment in solution, in the dry state as an impregnation of gauze, or in a cream-like paste, all these methods having proved efficient and useful.

In Solution. — At first, solutions containing as much as 4 per cent of chloramine-T were used in the treatment of wounds. Although there were no cases reported to show that these were unduly irritating, subsequent experience has shown that there is no occasion for exceeding a concentration of 2 per cent. For, as chloramine-T has, weight for weight, as great a germicidal power as sodium hypochlorite, a 2 per cent solution is considerably more potent than the

Medium	Concentration of Chloramine-T in the Mixture	Time of Action	Bacterial Count (1 Drop = $\frac{1}{10}$ cc.)
Blood Serum	1 : 1000	0	1751
		5 min.	0
		15 min.	0
		45	0
Blood Serum	1 : 2000	0	1831
		5 min.	9[1]
		15 min.	15
		45 min.	49
Blood Serum	1 : 3000	0	1509
		5 min.	96[1]
		15 min.	82
		45 min.	211
Peptone 0.7 per cent . .	1 : 5000	0	9360
		5 min.	0
		15 min.	0
		45 min.	0
Peptone 0.7 per cent . .	1 : 6000	0	1323
		5 min.	13[1]
		15 min.	12
		45 min.	14

solution of sodium hypochlorite usually employed (0.5 per cent). The 2 per cent solution may be used for the treatment of septic wounds, using the same methods as employed for the hypochlorites.

It must be borne in mind that in severe septic conditions much of the chloramine-T is promptly decomposed by the secretions. This is true of all antiseptics of the chlorine group and is even more marked in the case of hypochlorites than with chloramine-T. While this circumstance undoubtedly exerts a favorable influence through the destruction of substances of a toxic nature, it reduces the germicidal activity of the solution. It follows also that where sepsis is less marked, much weaker solutions may be employed.

[1] All antiseptic decomposed. No active chlorine present.

In the eye, for example, a solution of one part of chloramine-T in a thousand parts of normal saline solution will exert a satisfactory germicidal action, while 1 : 500 may prove rather irritating to the inflamed conjunctiva. Here, because of the constant irrigation by tears, there is chance for only a moderate accumulation of septic products likely to reduce the strength of the application. In cystitis, the tolerance often appears to be less than in the eye, and it is advisable to start treatment with a weak solution, increasing the strength according to the degree of tolerance manifested. In chronic urethral infections, 1 : 500 can be used for the initial injections and the concentrations subsequently increased. Similar considerations apply to the irrigation of the pleural cavity in empyema. The use of chloramine-T for the disinfection of meningococcus carriers is referred to on p. 100.

From the foregoing statements it will be evident that the choice of strength to be used must be left to the judgment of the surgeon. In practice it is advisable to keep a 2 per cent solution in stock and to dilute this, if necessary, either with water or, in case of considerable dilution, with normal saline solution. A 2 per cent solution is slightly hypotonic and when an approximately isotonic medium is desired, normal saline solution should be used as a diluent. Chloramine-T, like hypochlorites, has a corrosive action on most metals and should not be used for the sterilization of instruments.

In Impregnated Gauze. — Chloramine-T is well adapted to this use, for which very few substances of high antiseptic value have proved successful. It is possible, for example, to incorporate as much as 25 per cent of the weight of the gauze. This is a much larger amount than is advisable, 5 per cent being adequate. Obviously the gauze should not be moistened before use lest the antiseptic be washed out because of its ready solubility. It can be used dry for lightly packing and subsequently moistened if necessary

when in position. Impregnated gauze finds application in wounds of recent origin in which the chief object is to prevent progress of infection. It is particularly adapted to use in cases of industrial accident where treatment can be promptly instituted. Where frequent renewals of the antiseptic or irrigation are called for, it is superfluous.

In Soap Paste. — None of the chlorine group of antiseptics can be used in ointments containing fats or oils as these rapidly withdraw the active chlorine with the production of inert compounds. It is possible, however, with the exceptionally stable chloramine-T to obtain a preparation which can be used as an acceptable substitute for ointments. A preparation of this sort introduced by Daufresne,[1] which has been extensively used, contains 0.7 per cent to 1 per cent of chloramine-T dissolved in water containing 5–10 per cent of sodium stearate. It is important that the mixture should not contain any substance which can unite with chlorine to form an inert compound. For this reason the stearate used must be free from any fatty acid of an unsaturated series. The paste is not oleagenous. It has a creamy consistency and can be spread readily. As the antiseptic is dissolved in the water constituting the chief bulk of the paste, it has ready access to the parts treated.

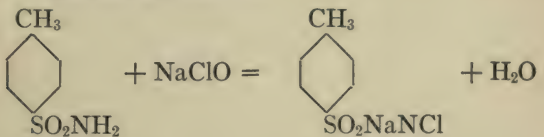
An investigation of the effect of this chloramine-T paste in sterilizing moderately infected wounds and maintaining asepsis in wounds previously sterilized by other means, has been published by Carrel and Hartmann.[2] Its use does not delay the rate of cicatrization.

Preparation. — Chloramine-T was first prepared by Chattaway[3] by the action of sodium hydroxide upon toluenesulphondichloramine (dichloramine-T, *vide infra*).[4] A more

[1] Journ. Exper. Med., *26*, p. 91, 1917.
[2] *Ibid.*, p. 95, 1917.
[3] Trans. Chem. Soc., *87*, p. 153, 1905.
[4] Chloramine-T is manufactured by Messrs. Boot, Island Street, Nottingham, England, and several other firms. It is also marketed by the Abbott Laboratories, Chicago, under the name of Chlorazene.

economical method of preparation consists in dissolving toluene-p-sulphonamide (1 mol.) in 5 per cent cold alkaline solution of sodium hypochlorite (1.2 mol.), warming gently if necessary, filtering, and adding $1\frac{1}{2}$ vols. of saturated salt solution. The chloramine-T crystallizes out of solution as a white glistening meal of crystals and is filtered off, washed with salt solution, and dried in the air. The product contains three molecules of water of crystallization. If it is desired to free the substance from adherent salt left from the process of preparation, it may be obtained pure from a hot concentrated solution, from which on slow cooling it will separate in large crystals. The purity of a given sample may be determined by titration with decinormal thiosulphate solution, as described on p. 42.

The reaction which takes place in the above preparation may be represented as follows:



Toluene-p-sulphonamide — Chloramine-T

Dichloramine-T

Dichloramine-T is the abbreviated name for toluene-p-sulphondichloramine. It is a yellowish white crystalline substance possessing a sweetish, rather pungent chlorous odor. It is stable in the solid state especially when kept in the dark. Water dissolves only traces of it, though it is readily soluble in most organic solvents except paraffin or petroleum. It has an intense germicidal action (pp. 86, 93) corresponding to its high content of active chlorine, but it is difficult to find perfectly satisfactory solvents for it which will yield stable solutions. Up to the present, the best

medium that we have been able to find is an oil obtained by the chlorination of paraffin wax, to which the name of "chlorcosane" has been assigned. Its preparation and properties will be described later (p. 37). Other solvents that have been used are mixtures of eucalyptol and paraffin oil both previously treated to reduce their avidity for chlorine as described on p. 39, and also a heavy oil (sp. gr. 1.2) obtained by prolonged chlorination of eucalyptol which was introduced by Lewis and Kraus. On the whole, "chlorcosane" appears decidedly preferable to the other solvents.

Dichloramine-T was originally used in oil solution for nasopharyngeal disinfection (p. 102), but more recently it has found a wider application in the treatment of infected wounds. The results obtained in the treatment of industrial injuries by W. E. Lee [1] and his colleagues in Philadelphia and by J. E. Sweet [2] in war wounds, have been extremely satisfactory.

It will be well, perhaps, to refer first of all to the way in which the antiseptic action of dichloramine-T in oil solution is exerted. It is well recognized that antiseptics incorporated with or dissolved in oily substances usually possess little if any antiseptic activity because intimate contact with the infected matter is hindered by the oil. When, however, such oil solutions of dichloramine-T as will be described are brought in contact with aqueous media, the partition coefficient between the oil and the water is such that a certain amount of dichloramine-T passes into the water and there exerts its germicidal action. The amount of dichloramine-T thus passing from the oil is enhanced by the presence in the aqueous medium of substances capable of taking up chlorine. So that the oil solution serves as a store of the antiseptic which is drawn upon to maintain the germicidal activity of the aqueous medium with which it is in contact. Thus the amount of active antiseptic leaving the oil solution is, to a

[1] Journ. Amer. Med. Assoc., July 7, 1917.
[2] Brit. Med. Journ., Aug. 25, 1917.

considerable extent, dependent upon the rate at which it is used up in the aqueous medium.

As illustrating the influence of varying conditions on the passage of the active chlorine from the oil to an aqueous medium, the following experiments may be cited. A 6.5 per cent solution of dichloramine-T, prepared as described later, was mixed (*a*) with an equal volume of saline, (*b*) with muscle extract, (*c*) with blood serum. The oil was then separated completely after 3 hours. The active chlorine stated in terms of dichloramine-T in the saline solution was 1 : 6000, in the filtered muscle extract 1 : 300, and in serum which was mostly coagulated 1 : 111. It is thus seen that the dichloramine-T dissolved in the oil is in a readily available form and direct bacteriological tests following its action on bacterial suspensions in blood and muscle extract (p. 86) on the organisms of the nasopharynx (p. 102) and on war wounds have clearly shown its germicidal action to be great.

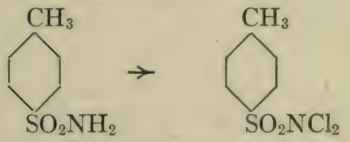
Dichloramine-T is best employed for surgical purposes in a 5–8 per cent solution in chlorcosane but it may also be used dissolved in eucalyptol and paraffin. The solution, however prepared, must be kept in amber glass bottles, since direct sunlight causes its rapid decomposition. Blue glass affords no protection. The solution is often most conveniently applied by means of an oil spray, an ordinary hard rubber or all glass atomizer being best, as some metals are slowly attacked. If the chlorcosane solution should prove too viscous for convenient spraying, about a tenth volume of carbon tetrachloride may be added. This addition should only be made to the oil used in a spray. The undiluted chlorcosane solution may be poured into wound cavities, and can easily be introduced into sinuses by means of a cotton swab dipped in the solution. The amount of the solution needed for each treatment is extremely small, 1 to 2 cc. being sufficient for most moderate-sized wounds, and it need not be renewed more often than once in 24 hours. A minimum of dressings

is required and they do not stick to the granulation tissue. Dichloramine-T, like most other antiseptics of the chlorine group, is an active lymphagogue when placed on fresh wounds. As granulation tissue develops, the lymph discharge decreases and the wound becomes comparatively dry. It also possesses the property of aiding in the removal of necrotic tissue. Wounds treated with the oil fill rapidly with granulation tissue of healthy color which shows no tendency to exuberant growth nor to become sodden. The early reports as to the results of the use of dichloramine-T in oil solution for wound treatment are decidedly encouraging and the method appears to us to be of genuine value and deserving of extended use.

Dichloramine-T in oil solution has been tried extensively in the early treatment of injuries, not only of the soft parts but of tendons, bones, and joints. At the primary dressing of these wounds, after the excision of dead tissue and obvious foci of infection, the wound is liberally flooded with the oil and then closed without drainage. In Lee's experience primary union follows in at least 75 per cent of these sutured wounds if treated within three hours of their infliction. If signs of infection should appear, one or more stitches are removed and oil is introduced by means of a grooved director to the focus of infection once in 24 hours. With wounds treated after a longer interval the question of their closure becomes a matter of surgical judgment. Dichloramine-T in oil has also been found decidedly useful in the treatment of boils and carbuncles with minimal incision, in osteomyelitis, non-tuberculous empyema and in the control of postoperative wound infection. More recently it has been used by Lee for the treatment of burns, and in these cases it is advisable to cover the tissues with one layer of a coarse-meshed gauze or mosquito netting previously soaked in melted paraffin wax. In this way the exudate easily comes through the open mesh and the dressings do not stick. It is an advantage if such wounds can be exposed to the air.

The absence of suppuration and freedom of drainage in these cases is significant.

Preparation of Dichloramine-T. — The following details are based on the method employed by Chattaway: Bleaching powder (350 to 400 gms.) of good quality (25 per cent or more "available chlorine") is shaken with two liters of water in a shaker for an hour and then the mixture allowed to settle. The supernatant fluid is siphoned off and the remainder filtered. Powdered toluene-sulphonamide (75 gms.) is then added to the whole of the hypochlorite solution and shaken till dissolved. The solution is filtered if necessary, placed in a large separating funnel, and acidified with acetic acid (100 cc.) added in portions. About 100 cc. of chloroform are then added to extract the dichloramine precipitated by the acid, and the whole well shaken. The chloroform layer is tapped off, dried over calcium chloride, filtered, and allowed to evaporate. The residue is powdered and dried in vacuo. It is sufficiently pure for most purposes without recrystallization. The yield is practically theoretical. The product may be tested by titration with thiosulphate solution (p. 42). Its preparation may be represented as follows:



p-Toluenesulphonamide Dichloramine-T

Preparation of Solvents for Dichloramine-T. — *Chlorcosane.* — This is at present the most satisfactory solvent for dichloramine-T and is prepared as follows: Paraffin wax, preferably melting at 50° C., or higher, is placed in round bottomed flasks and heated to about 120°. Two flasks connected in series, each containing a half kilo of the wax, may be conveniently used. A rapid current of chlorine

from a cylinder of the liquefied gas is then passed through the molten wax in the flasks, each of which is provided with a thermometer and the necessary glass tubes. The temperature should be controlled within the limits of 125–140°. The first flask in which the reaction is most vigorous will require but little heating. Chlorination is continued until the contents of the flasks have increased in weight 45–55 per cent of the weight of wax taken. It will be found convenient to complete the chlorination of the first flask and then remove it, and transfer the second to its place, putting a fresh flask of paraffin wax after it. In order to avoid undue discoloration of the product, a minimum amount of rubber tubing should be used for the connections. Wide glass tubing should be used for passing the gases, and the ends of the delivery tubes are preferably blown into small bulbs provided with a number of fine orifices to promote good contact between the gas and oil. Hydrochloric acid is of course evolved freely during the reaction. After the requisite amount of chlorine has been absorbed, the oil while still warm is shaken vigorously with five per cent of its weight of dry sodium carbonate and then filtered through a dry fluted paper. The clear oil, which has a light yellow or sherry color and is slightly heavier than water, is then ready for use. It possesses a viscosity intermediate between that of olive oil and castor oil, has almost no odor, and is perfectly bland when sprayed up the nose or placed on skin or wounds. It will be noted that the materials necessary for making the solvent are all easily available at very cheap rates. As was to be expected, the product is not a single individual compound, but a mixture of isomeric and homologous chlorine derivatives. It is therefore impossible to give a systematic name to the product, and as the descriptive name "chlorinated paraffin wax oil" seems unnecessarily clumsy, we have chosen the name "chlorcosane" since the systematic names of most of the hydrocarbons in paraffin wax end with the suffix "cosane." Chlor-

cosane itself contains all its chlorine united to carbon and has no appreciable antiseptic action.

Chlorinated Eucalyptol. — Eucalyptol (U. S. P. or Brit. Pharm.) and not eucalyptus oil, must be used. Five hundred cc. are treated with 15 gms. potassium or sodium chlorate and 50 cc. concentrated hydrochloric acid for 12 hours or longer. It is then well washed in a separating funnel, first with water and then with a solution of sodium carbonate, to remove all traces of hydrochloric acid. After tapping off the aqueous layer, 15 gms. dry sodium carbonate are added to the oil and the whole allowed to stand for 24 hours. It is then filtered, further dried with a little solid calcium chloride, which must be allowed to act for a considerable time, and is then ready for use.

Chlorinated Paraffin Oil. — This may be used for diluting solutions of dichloramine-T in eucalyptol, or other solvents. Paraffin oils derived from different sources vary greatly in their capacity for taking up chlorine. Russian oils containing much naphthenes and some American oils containing considerable quantities of olefines are unsuitable. The best oils with which we are acquainted are those from Pennsylvania which have been well purified with fuming sulphuric acid and then washed and dried. The chlorination of the oil may be conveniently effected as just described for the eucalyptol. It will be found advisable to shake the chlorinated oil with about one per cent of animal charcoal before finally filtering. Paraffin oil itself whether chlorinated or not only dissolves insignificant traces of dichloramine-T.

Preparation of Dichloramine-T Solutions. — The constituents of the solutions are all stable and may be preserved indefinitely especially in colored bottles. To dissolve dichloramine-T in chlorcosane it is convenient to warm about a quarter of the oil to 75° or 80°, add the dichloramine-T which will promptly melt, and then stir in the remainder of the cold oil. An eight per cent solution thus prepared and

kept in amber bottles, protected from excessive heat, will still contain over seven per cent at the end of a month. An accumulation of crystalline deposit, toluene sulphonamide, in the solution is evidence of decomposition and such solutions should be rejected. Careful protection from unnecessary exposure to light, heat, or moisture will do much to prevent decomposition. If instead of chlorcosane, eucalyptol be used as solvent, it is advisable to dissolve the dichloramine-T (10 grams) in chlorinated eucalyptol (75 cc.) and then add chlorinated paraffin oil (75 cc.). This mixture contains about 6.5 per cent of dichloramine-T. The proportion of paraffin may be reduced one half if a stronger solution is required.

The Chemical Determination of the Concentration of Chlorine Antiseptics

It is very desirable that the concentration of solutions of the various antiseptics of the chlorine group should be subjected to analytical control. This is particularly true in the case of the hypochlorites and other unstable products.

In principle, the same method is used for all the substances mentioned in this chapter. A known quantity of the solution or substance is taken and an excess of potassium or sodium iodide and of acetic acid is added. Iodine is at once liberated in amount equivalent to the active chlorine of the antiseptic, and this iodine is measured by determining the amount of a decinormal solution of sodium thiosulphate necessary to react completely with it. The following solutions are required:

Decinormal Sodium Thiosulphate Solution. — This is prepared with sufficient accuracy by dissolving 24.8 grams of the pure crystals in water and diluting to 1000 cc. The solution is moderately stable, especially if protected from light, and will serve for two or three months.

Each cubic centimeter of this solution is equivalent to:

0.0127 gram Iodine
0.00354 gram Chlorine
0.00262 gram Hypochlorous Acid
0.00372 gram Sodium Hypochlorite
0.01407 gram Chloramine-T
0.006 gram Dichloramine-T

Iodide Solution. — A 10 per cent solution in water.

Acetic Acid. — A 10 per cent solution of the pure acid.

Starch Paste. — Prepared by boiling about 0.1 gram starch with 100 cc. of water, cooling, and allowing to sediment. The clear solution is used as an indicator for iodine.

Determination of the "Available Chlorine" in Bleaching Powder. — A fair average sample from bulk is taken and of this 10 grams is exactly weighed out into a mortar. The powder is triturated in the mortar with successive small quantities of water and completely transferred to a liter flask which is filled to the mark with water. The whole is well shaken and allowed to stand for an hour or two. Ten cc. of the supernatant liquid is measured with a pipette and transferred to a small flask. Five cc. each of the iodide and acetic acid solutions are then added. The iodine which is now liberated is determined by adding from a burette the decinormal sodium thiosulphate solution until almost all the iodine has disappeared. A few drops of the starch paste are then added and the addition of the thiosulphate continued until the blue color just disappears. The 10 cc. of bleaching powder solution is equivalent to 0.1 gram of the solid substance and as each cc. of thiosulphate = 0.00354 gram available chlorine, the percentage of available chlorine in the bleaching powder is found by multiplying the number of cubic centimeters of thiosulphate used by $0.00354 \times 1000 = 3.54$. Thus if a particular sample of bleaching powder treated as described required 9.4 cc. of N/10 sodium

thiosulphate, the available chlorine would be 33.3 per cent.

Titration of Sodium Hypochlorite Solutions or Eusol. — Ten cc. of the solution is treated with 5 cc. each of the iodide and acetic acid solutions and then titrated with sodium thiosulphate, as described above. Each cubic centimeter of thiosulphate used represents 0.00372 gm. sodium hypochlorite or 0.00262 gm. hypochlorous acid. The quantity of hypochlorite or hypochlorous acid in 100 cc. will be given by multiplying the number of cubic centimeters of thiosulphate used by 0.0372 or 0.0262 respectively.

Estimation of Chloramine-T and Dichloramine-T. — These substances are examined for their content of active chlorine, as described for the hypochlorites with the slight difference that in order to facilitate the reaction of the chloramines with the iodide, it is well to add a little chloroform (5–10 cc.) or carbon tetrachloride before titrating. The method of calculation follows from the fact that each cubic centimeter of thiosulphate used in the titration is equivalent to 0.01407 gm. crystallized chloramine-T or 0.006 gm. dichloramine-T. It will be noticed that one molecule of chloramine-T liberates two atoms of iodine and one molecule of dichloramine-T liberates four atoms of iodine. The reason for this is that each atom of chlorine in the antiseptic is equivalent to a molecule of hypochlorous acid, each of which liberates two atoms of iodine from an acidified iodide solution.

$$HClO + 2\,HI = I_2 + HCl + H_2O$$

CHAPTER III

THE PHENOLIC GROUP OF ANTISEPTICS

MANY of the common antiseptics belong to this group and have been long employed for routine disinfection both for surgical and hygienic purposes. It cannot be said that any new properties of value have been observed in this group as the result of their employment in war surgery.

Phenol has found steady advocates ever since Lister adopted it as an aid in obtaining his brilliant successes in antiseptic surgery. Early in the present war the mistaken notion seemed to prevail that a decision as to the general utility of antiseptics could be attained by treating infected wounds receiving a minimum of preliminary surgical treatment with pure phenol and observing whether infection supervened. In the light of present experience it seems hardly necessary to say that wound sterilization cannot often be effected by such methods. The use of destructive coagulants such as phenol in high concentration is undoubtedly undesirable and apt to lead to conditions favorable to the growth of anaërobes. On the other hand, phenol at a concentration of 2.5 to 5 per cent is still in common use and a mixture of equal parts of one of these solutions with hydrogen peroxide is viewed with favor by many. Phenol and camphor when rubbed together in equal proportions give a liquid which is said to give useful results in infected cases of long standing.[1]

[1] Feldman and Walton, Lancet, Dec. 3, 1916.

The stability of phenol solutions and their clean odor are attractive qualities but, while phenol is undoubtedly a good disinfectant for many purposes, it does not seem to give as good results in the treatment of badly infected wounds as many other antiseptics. The use of alcohol and glycerine [1] as solvents for phenol has not much to recommend it, viewed simply as regards disinfection, since both of these solvents depress its germicidal activity. Phenol dissolved in vegetable oils is almost devoid of germicidal activity as but little of the antiseptic leaves the fatty solvent. Much weaker solutions dissolved in mineral oil, in which it is sparingly soluble, have been found by Lewis and Richards to be more effective. The fairly strong inhibitory effect of phenol upon phagocytosis has already been referred to, although it may be doubted whether this is as important a matter as it is sometimes regarded.

Anthrax spores are remarkably resistant to phenol solutions and may be viable after four days' immersion in a five per cent solution. When acting in the presence of blood serum, defibrinated blood, or pus, for reasonable lengths of time, *e.g.* 2 hours, concentrations below 2 per cent are relatively ineffective against pyogenic cocci. The rather slow but progressive disinfection of a heavily infected mixture of blood serum and muscle extract, to which one third volume of 2 per cent phenol had been added, is well illustrated on p. 86. Sterilization was incomplete after 22 hours although less than 1 per cent of the organism survived. The way in which phenol exerts its bactericidal action is not understood. Cooper [2] has produced some evidence tending to show that chemical reaction between the bacterial proteins and phenol is not a *sine qua non* of phenol disinfection and it is suggested that the absorption of phenols by bacteria is merely the initial stage in the pro-

[1] Goodrich, Brit. Med. Journ., May 19, 1917.
[2] Biochem. Journal, 7, p. 175, 1913.

cess of disinfection and that the germicidal action which follows is due to a de-emulsifying action upon the colloidal suspension of some constituent protein essential for the stability of the organism. With phenol and its derivatives there appears to be an intimate relation between their germicidal powers and their protein precipitating capacity.

Phenol is commonly used as a standard for the measurement of the efficiency of disinfectants by the Walker-Rideal method (page 79).

Various halogen derivatives of phenol have been proposed as antiseptics and while many of them are highly germicidal against bacteria suspended in water, they are not particularly active in the presence of blood serum or other protein material and hence have little to recommend them for wound treatment. A great many of these compounds have been carefully studied by Bechold and Ehrlich.[1]

Cresols, or methylphenols, occur in three isomeric modifications. They are more actively germicidal than phenol itself and it is stated that a 1 per cent solution of commercial cresol in water is as active as 3 per cent phenol. The commercial mixture goes by the name of "tricresol" and is often employed for sterilization of the hands and of instruments. Cresol paste, made with lanoline and white wax, was recommended by Sir. W. Watson Cheyne [2] and his colleagues for the early treatment of infected war wounds, but the results obtained early in the present war were generally regarded as unfavorable and its use has been discontinued.

Lysol is prepared by treating the fraction of tar-oils chiefly composed of cresols with fat and then saponifying with alcoholic soda. Its use for general disinfecting purposes is well known, but its employment as a dressing for wounds is limited, except in veterinary practice. It gives

[1] Zeitschr. f. physiol. chem., *47*, p. 173, 1906.

[2] Journ. Royal Naval Medical Service, April, 1915. Lancet, Nov. 21, 1914, Feb. 27, 1915.

a soapy, frothing solution when mixed with water. *Creolin* is a similar preparation.

The interesting suggestion has been made by Miss Mary Davies[1] that wound infections might be limited to some extent by the use of antiseptic substances for impregnating the clothing of soldiers. After studying the effect of various substances she concludes by recommending the use of a 5 per cent solution of "pyxol," a cresol and soft soap preparation analogous to lysol. It appears that some bactericidal power is retained by cloth so treated after a month's exposure to sun and rain and possibly even for a longer period. The practical results of these suggestions will be awaited with interest.

Thymol, or propylmethylphenol, has been recommended as an antiseptic for surgical purposes but has been practically discarded, although dentists find its low solubility an advantage in some conditions requiring antiseptic treatment. *Di-iododithymol* is known under the name of *aristol*. It is used to some extent as a substitute for iodoform but is unsuited for general use in the treatment of war wounds.

Salicylic Acid, or orthohydroxybenzoic acid, has received much attention as an antiseptic for the treatment of war wounds. In particular, a powdered mixture of salicylic acid and boric acid, introduced by Sir W. Watson Cheyne[2] under the name of *borsal*, was given an extended trial. A mixture of salicylic acid and borax had been tried, with ineffective results, as a wound dressing by the Japanese in the Russo-Japanese war. Borsal does not seem to have achieved much greater success in most surgeons' hands, and after fairly extensive trials in France, either alone or in conjunction with cresol past, its use was abandoned.[3] It appeared that borsal did not effectively

[1] Lancet, Sept. 30, 1916, p. 603.
[2] Brit. Med. Journ., May 22, 1915, p. 912.
[3] *Ibid.*, June 5, 1915, p. 984.

check sepsis save in superficial wounds and its use, in the opinion of many, was not unattended by danger. It seems that the coagulating action of salicylic acid on blood and wound exudates impedes free drainage and under these conditions, when sterilization has not been complete, the possibilities for the growth of anaërobes such as the gas bacillus and bacillus of malignant œdema, are considerable. The disinfecting action of salicylic acid alone is not great in the presence of wound exudates and is, moreover, limited by its low solubility, 1 : 500 in cold water. Addition of borax causes a much larger quantity of salicylic acid to dissolve owing to the formation of a double salt, sodium borosalicylate, which is freely soluble. Solutions of this salt are used to a small extent as antiseptic lotions. Alcoholic solutions of salicylic acid have been added in small quantity to the last funnel full of saline used for irrigating septic wounds.[1] In this way the salicylic acid is precipitated by the water and may be evenly distributed over the surface of the wound. This method has been found to be preferable to dusting the dry powder on the wound.

A large number of halogen derivatives of salicylic acid have been examined without revealing any particularly valuable qualities, although occasionally their germicidal properties are found to be markedly greater than those of salicylic acid.

β-Naphthol and Bromonaphthols. — The naphthols have antiseptic properties similar to those of the simpler phenols but have not been used extensively as wound antiseptics, although *β*-naphthol finds some employment as an intestinal disinfectant and is regarded as valuable in ointments for the treatment of skin diseases of parasitic origin. Recently *β*-naphthol has been used as an antiseptic addition to the paraffin wax mixtures used in the treatment of burns. The composition of "ambrine," one of the best known of these

[1] L. Garret Anderson and Helen Chambers, Lancet, June 3, 1916.

mixtures, has not been disclosed by its proprietary owners but a product made according to the following formula, due to A. J. Hull,[1] is stated to give equally good or better results: β-naphthol 0.25 per cent, eucalyptus oil 2 per cent, olive oil 5 per cent, hard paraffin 25 per cent, and soft paraffin 67.75 per cent. The mixture may be applied with a broad camel hair brush or sprayed on at a temperature of about 50° C. An illustration of the form of spray used for this purpose in the naval service will be found in the British Medical Journal, August 28, 1917. *Resorcinol*, 0.25–1 per cent, may be used in place of the β-naphthol. The preparation of the mixture is as follows: Melt the hard paraffin, and add the soft paraffin and olive oil. Add the resorcinol dissolved in half its weight of absolute alcohol and lastly add the eucalyptus oil when the wax has cooled to about 55°C.

Becholdt[2] has carried out experiments with a series of bromine derivatives of β-naphthol and finds that several of them have germicidal properties of a high order when tested against pyogenic cocci in water suspension. The tribrom-β-naphthol in particular was found to be especially active, killing staphylococci at a dilution of 1 : 250,000. Our own experiments have, however, indicated a materially lower germicidal value. This substance has been made commercially and endorsed as an efficient antiseptic agent for the treatment of infected wounds. It is, however, not suitable for military surgery as it is very sparingly soluble and is not significantly more active in the presence of blood serum than ordinary β-naphthol. When dissolved in alcohol and tested against staphylococci suspended in serum, the lethal concentration is reduced to 1 : 800 or less.

Picric Acid (trinitrophenol). — Picric acid is made use of more for the treatment of burns than for ordinary infected

[1] Brit. Med. Journ., Jan. 13, 1917.
[2] Zeitschr. f. Hyg. u. Infekt. Krankh., *64*, p. 113, 1909. Zeitschr. f. Angew Chem., *22*, p. 2033, 1909.

wounds. The acid is soluble at room temperature in about ninety parts of water and dissolves much more readily in alcohol or ether. A 1 per cent or saturated aqueous solution is generally employed for surgical purposes. As a first dressing for burns it has proved of the greatest value and does much to relieve pain and reduce the risk of subsequent infection. It is employed either in solution or as impregnated gauze or wool.

The use of picric acid in the treatment of extensive wounds is probably to be deprecated, not only because other more suitable antiseptics are available but also because the substance is decidedly toxic. The germicidal properties of picric acid tested against staphylococci or *B. coli* in aqueous media are moderately high; using the Walker-Rideal method of testing, it is found to have a "phenol coefficient" variously estimated between 4 and 6.[1] But picric acid is an active protein precipitant and it is unlikely to be capable of exerting very much germicidal action in the presence of serum or wound exudates. Direct experiments in this point appear to be lacking. The coagulating and hardening effect of picric acid due to the power of precipitating proteins is probably responsible in part for its successful use in the treatment of burns.

[1] H. L. Tidy, Lancet, Sept. 11, 1915.

CHAPTER IV

SALTS OF THE HEAVY METALS AS ANTISEPTICS

THE metallic salts which are used because of their antiseptic properties are mainly those of silver, mercury, bismuth, and zinc. With one exception, namely, Rutherford Morison's bismuth paste, no very extended use of these substances has been made in the present war. In the following chapter, their mode of action will be referred to first of all, then their germicidal effects and, lastly, some of their applications.

The germicidal activity of many of these metallic salts, when acting upon bacteria suspended in pure water, is extraordinarily high. But this powerful action is enormously reduced as soon as the bacteria are placed in other media than pure water. Most of the soluble salts of these metals, with the exception of some colloidal preparations, suffer from the disadvantage that they are precipitable by proteins or some of the constituents of wound exudates such as phosphates, so that their high initial antiseptic potency is soon reduced. It also follows from this fact that the metallic salts find their most useful applications under conditions which do not lead to their rapid precipitation, and indeed under such circumstances they are among the most valuable disinfectants.

There is a good deal of evidence pointing to the belief that the metallic ions present in aqueous solutions owing to electrolytic dissociation are the chief disinfecting agents [1] and

[1] Dreser, Arch. f. Exper. Path. u. Pharm., *32*, p. 456, 1893.

that the undissociated salts as such are of minor importance. Thus Krönig and Paul found that the disinfecting action of equimolecular quantities of mercuric chloride, bromide, and cyanide was in proportion to their ionic dissociation in solution. Miss Chick has shown that the laws found to govern other examples of disinfection are only applicable to the action of mercuric chloride if the concentration of Hg'' ions is used as the basis of calculation rather than the total concentration of the salt. The metallic ions are responsible for the ordinary chemical reactions of metallic salts in aqueous solution and those metallic compounds which do not yield the ordinary chemical reactions for the metals, such as many protein and other colloidal preparations of the metals, possess inferior disinfecting properties. An interesting example of the importance of the metallic ions in disinfection is shown by the following observation: Two silver salts were compared as regards their action on staphylococci in water and blood serum. One of these salts, silver fluoride, which undergoes dissociation with formation of silver ions, killed in two hours staphylococci in water at a concentration of less than 1 : 10,000,000. The other salt was the double cyanide of silver and sodium which, on solution in water, gives few if any metallic ions but remains in solution as a complex aggregate. When tested against staphylococci in water, the lethal concentration was only about 1 : 5000. Thus it will be seen that silver fluoride solution containing metallic ions is, under the conditions of the experiment, two thousand times as active as silver sodium cyanide which gives few or no metallic ions. When the same salts were tested against staphylococci suspended in blood serum, the lethal concentrations were much more closely approximated, being about 1 : 7000 and 1 : 3000 respectively. It appears probable that the metallic ions of the salts exert their disinfecting action by reacting chemically with the protein or other constituent of the

bacterial protoplasm, and indeed in some cases this phenomenon has been actually observed. Salts of the heavy metals, even when present in a nutrient medium in extremely small amount, are capable of exerting an inhibitory influence upon the growth of bacteria, so that in all estimations of the germicidal action of these salts it is essential to avoid carrying over any of the antiseptic into subcultures. In addition to the inhibitory action upon the growth of bacteria exercised by traces of metallic salts, Miss Chick [1] has noted another phenomenon exhibited by this class of disinfectant. "If bacteria are subjected to the action of 1 : 1000, 1 : 10,000 or even weaker solutions of mercuric chloride, there is an interval during which some at least of them may be resuscitated by the timely administration of an antidote (in this case a sulphide solution), but if this antidotal treatment is not employed, no amount of subsequent dilution beyond the limits when inhibition occurs, can prevent the death of the organism. It would seem that the mercuric salt has been already absorbed by the bacteria and possibly formed some combination with its substance, not however to a sufficient extent to prevent recovery if a large excess of the sulphide solution be employed." In one case it was observed that in a 24-hour culture of *B. paratyphosus* some individuals at least were able to manifest vitality after contact with 5 per cent mercuric chloride solution for four minutes, provided ammonium sulphide was promptly applied as antidote.

There is an extensive literature dealing with the germicidal action of the metallic salts, but most of the results are not comparable with one another owing to variations in the technique employed in making the tests. Even slight variations in the composition of the medium in which these substances act produce enormous variations in the results of the experiments. Thus we have found the apparent activity of mercuric chloride against staphylococci in a

[1] Journ. of Hygiene, *8*, p. 92, 1908.

hard tap water to be only one fifth of that shown when distilled water was employed, while with silver salts the results are even more liable to variation. The following extracts from published experiments are given simply as a rough guide to antiseptic potency, but for details, the original papers must be consulted.

The use of mercuric chloride as a disinfectant practically dates from Robert Koch's [1] experiments published in 1881. Somewhat later, Geppert showed that Koch had overestimated the antiseptic value of the salt through error in technique involving the carrying over of mercury salts into subcultures. Geppert's experiments were adversely criticized by von Behring [2] but have been essentially substantiated by subsequent workers. The action of mercuric chloride upon spores, especially those of *B. anthracis*, has been most carefully studied by Krönig and Paul,[3] Madson and Nyman,[4] and Miss Chick.[5] A chart from Miss Chick's paper, in which the figures are taken from Krönig and Paul's experiments, gives a good idea of the general character of the results, though many more details will be found in the original papers.

As exemplifying the action of mercuric chloride on a vegetative form, *B. paratyphosus*, the following chart is reproduced from Miss Chick's paper.

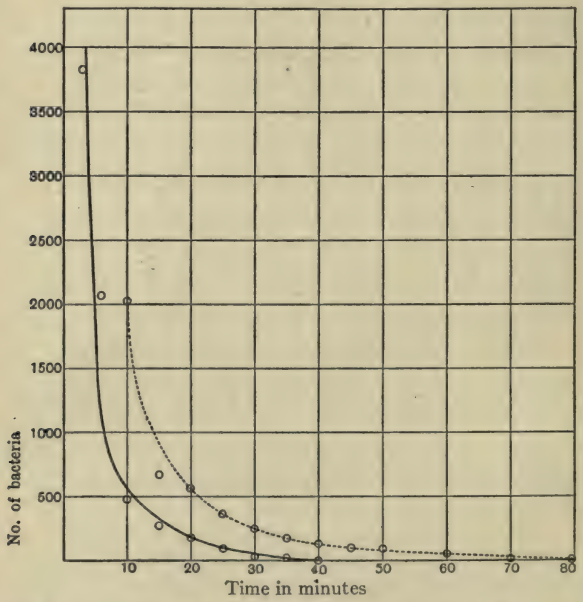
The action of mercuric chloride upon bacteria is not nearly so rapid as is commonly thought to be the case. Miss Chick found that *B. paratyphosus* could withstand the action of five per cent bichloride for four minutes, and *staphylococcus aureus*, for fifteen minutes, if at once treated with a sulphide antidote.

But these experiments relate only to the action of the antiseptic on suspensions of organisms in an aqueous medium.

[1] Ueber Desinfection, Mittheil. Kaiserl. Gesendheitsamt, Vol. I.
[2] Zeitschr. f. Hygiene, *9*, p. 396, 1890.
[3] *Ibid.*, *25*, p. 1, 1897.
[4] *Ibid.*, *57*, p. 388, 1907.
[5] Journ. of Hygiene, *8*, p. 92, 1908.

When working in a blood serum medium, its activity is much reduced, while in the presence of whole blood, pus, or muscle

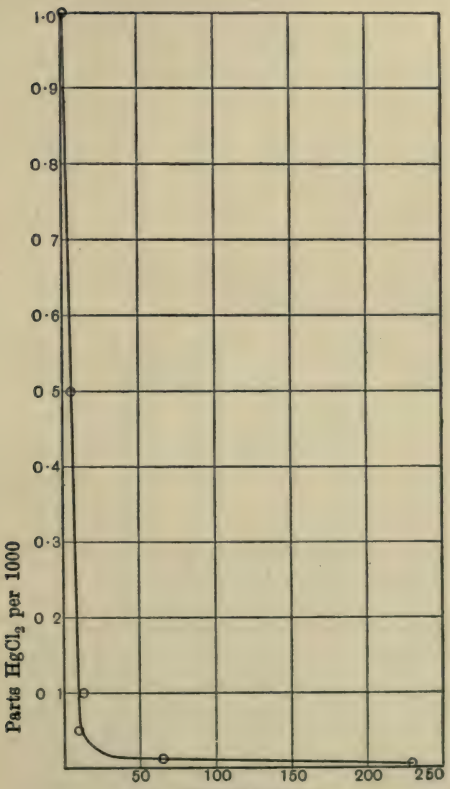
CHART I



Illustrating the results of Krönig and Paul's experiment. Continuous curve, disinfection of anthrax spores with 2.1 per 1000 $HgCl_2$. Dotted curve, disinfection of anthrax spores with 1.1 per 1000 $HgCl_2$.

extract much higher concentrations are required for effective action. A single new experiment may be quoted to illustrate this (see p. 87). Mercuric chloride (1 cc. 1 : 1000) was added to blood serum (1 cc.) and 50 per cent muscle extract (1 cc.) previously heavily inoculated with

CHART II



Times taken for disinfection of *B. paratyphosus* with varying concentrations of mercuric chloride, H_2S being used as antidote.

staphylococcus aureus. The final concentration of mercuric chloride was therefore 1 : 3000. The number of surviving organisms was estimated at varying intervals of time.[1] The experiment was conducted at 32° C. Although at the end of five minutes almost 90 per cent of the organisms were killed, the mixture was not sterilized completely at the end of three hours.

Among other salts of mercury that are employed for surgical purposes, are mercury potassium iodide, cyanide, oxycyanide and double zinc cyanide. "Mercuric biniodide," *i.e.* mercuric potassium iodide, resembles mercuric chloride closely in germicidal properties but is regarded as less irritating. The other salts mentioned are less active under most conditions.

The germicidal action of most silver salts closely resembles that of the corresponding mercuric compounds. Silver chloride is insoluble and hence ineffective, but silver nitrate appears to resemble mercuric chloride fairly closely. Its action on *B. paratyphosus* has been quantitatively studied by Miss Chick with results that resemble the curve given on p. 55. Silver cyanides, colloidal silver, and various organic compounds which yield few silver ions on solution in water all appear to have inferior germicidal properties, although some of them find useful application in civil practice. For their preparation, properties, and uses, reference must be made to textbooks of pharmacology.

Experiments on the action of silver nitrate solution and argyrol on considerable quantities of staphylococci and other organisms suspended in equal parts of blood serum and muscle extract are recorded on p. 87. Silver nitrate (1 per cent) added so that the final concentration was 0.33 per cent killed about 95 per cent of the organisms in six hours but failed to sterilize completely in 24 hours. Argyrol with a final concentration of 5 per cent did sterilize

[1] Potassium sulphide was added as "antidote" to prevent the carrying over of active mercury salts to the subcultures.

in 24 hours but many organisms were still present at the end of six hours.

The salts of bismuth do not appear to have been examined very carefully as regards their bactericidal action, although their action in checking undesirable fermentations in beer worts was discovered long ago. Most normal bismuth salts are more or less rapidly decomposed by water with formation of insoluble basic salts, and some of these have found effective use as mild antiseptics capable of slow but prolonged action.

Zinc salts have long been known to have antiseptic properties and the chloride especially has been used by Lister, Kocher, and others. Its germicidal action is far inferior to that of most mercury or silver salts. A 5 per cent solution is ineffective against anthrax spores but a 2.5 per cent solution is reported effective against most vegetative forms in a reasonably short time, when acting in an aqueous medium. Its activity is however much influenced by the medium in which it acts, since it is very readily precipitated by proteins, phosphates, etc. An experiment in which 3 per cent zinc chloride was added to a mixture of equal parts of blood serum and muscle extract inoculated with staphylococci and other organisms, so that the final concentration of zinc chloride was 1 per cent, showed that about one sixth of the organisms survived at the end of an hour and a half and that complete sterilization was not quite accomplished after 24 hours (p. 88).

Uses of Metallic Salts in War Surgery. — The practical uses of the metallic disinfectants in the treatment of infected war wounds has not been extensive with the exception of a paste containing bismuth subnitrate which will be referred to later. Mercury salts alone have almost no advocates, although a certain amount of gauze impregnated with Lister's double cyanide of mercury and zinc is still employed, with the object of preventing re-infection of the wound

and in the hope of controlling further progress of the existing infection. A preparation of mercuric chloride and malachite green introduced by Fildes, Rajchman, and Cheatle has, however, given useful results, especially in chronic suppurating wounds. An account of this preparation will be found in the section on dyes (p. 61).

Silver nitrate at 1 : 1000 was tried for some time, but the darkening of the treated wounds on exposure to light was a drawback and the results appeared to be only moderately good, so that at the present time it is scarcely used at all.

Zinc salts have a marked caustic and coagulant action and, as is well known, are frequently employed on account of these effects on torpid ulcerations, fistulous tracts, etc. Lister long ago made use of zinc chloride solution in many infected conditions and noted the fact that the precipitation of zinc compounds in the coagulated surface of the wound was generally sufficient to prevent recurrence of sepsis. Strong solutions of zinc chloride, up to as high as 10 per cent, were believed useful, particularly by Belgian surgeons, when used as wet dressings on wounds which had been freely incised subsequent to the development of gas gangrene infection. This treatment, which is a drastic one, has been largely supplanted by irrigation with some form of hypochlorite solution.

By far the most useful metallic salt antiseptic so far employed in the present war is the bismuth paste introduced by Rutherford Morison.[1] This is made by mixing bismuth subnitrate (1 part) and iodoform (2 parts) with sufficient liquid paraffin oil to make a thick paste of such consistence that it may be readily spread in a thin layer with the help of a spatula or spoon. This mixture, which is commonly known as "B. I. P.," was first of all recommended for the treatment of infected war wounds in which suppuration was already established. But it is now used to a con-

[1] Lancet, Aug. 12, 1916, p. 268.

siderable extent for the treatment of fresh wounds, partly owing to its ease of application and the fact that frequent redressing is usually unnecessary, although adequate provision must be made for free drainage. When casualties are unusually numerous and speed of treatment becomes of great practical importance, the paste is said to be particularly useful.

The method of using the paste for suppurating wounds is essentially as follows: After appropriate surgical treatment, the wound cavity and surrounding skin is carefully mopped with alcohol. The wound is then filled with the paste, and dressed with gauze which is covered with an absorbent pad, which in turn is held in position with sticking plaster and a bandage. This dressing requires no change for days or weeks if the patient is free from pain and constitutional disturbances. Should, however, discharge come through the dressing, the stained part must be soaked in alcohol and a fresh gauze dressing, wet with alcohol, applied as a further covering. In order to redress the wound, wool soaked in alcohol is used to wipe away the sticky, dirty looking discharge. The wound and a small area of skin is again plastered with paste and this in turn covered with gauze dressing, pad, and bandage, as before.

It is claimed that the results following the use of this paste are notably good in fractures of the long bones, and the ease of dressing as well as its infrequency and the absence of pain are valuable features.

Precise bacteriological analysis of the effects of the paste upon infected wounds has not yet been supplied, so that final judgment as to the bactericidal value of the mixture must be delayed. It must not be forgotten that the constituents of the mixture are not innocuous and that iodoform poisoning, particularly when the drug is placed in closed cavities, is not uncommon. However, iodoform poisoning seems to be very rarely encountered with the present mixture.

The paste appears to be very slowly absorbed and may cause disturbance long after apparent healing of the wound. A small sinus may form and iodoform suspended in a brownish fluid may escape, but the exit wound heals promptly. In the majority of cases, however, it is slowly absorbed and by means of X-rays the slow disappearance of B. I. P. in bone cavities, etc., can be readily followed.

CHAPTER V

DYES AS ANTISEPTICS

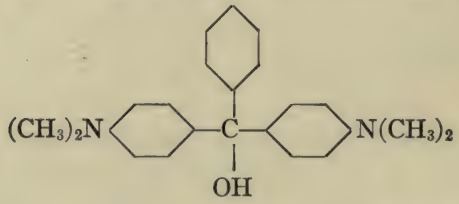
A LARGE number of dyestuffs possess germicidal properties, although until recently they have been employed for the destruction of blood parasites such as trypanosomes rather than bacteria.

Malachite green, used in conjunction with mercuric chloride, was recommended early in 1915 in a report to the Medical Research Committee, by Fildes, Rajchman, and Cheatle,[1] and has been used fairly extensively, especially in the naval service. A 2 per cent solution of malachite green in 80 per cent pure alcohol is mixed with an equal volume of a 2 per cent solution of mercuric chloride in 80 per cent alcohol. The two solutions are best kept apart until needed for use. The mixture, as Micklethwaite has shown, contains a double compound consisting of one molecule of malachite green and two molecules of mercuric chloride. This compound appears to be readily dissociated in contact with the tissues. The malachite green is reduced by living tissues to the leuco-compound and therefore becomes invisible in a wound but may still maintain its activity. Sloughs and necrosed tissue, however, do not reduce the dye. The mixture is generally applied by means of a spray and is surprisingly non-irritating when the concentration of the mercury salt is considered. It has been found particularly useful as a skin-disinfectant and for the treatment of superficial wounds, but it has also proved of value in cases of osteomyelitis, septic

[1] Lancet, 1915, ii, p. 165.

fractures, and burns. Experiments on the germicidal effect of malachite green when acting on organisms either in a blood medium or blood serum-muscle extract (p. 89) lead us to the conclusion that the value of the dye as a wound antiseptic probably has been much overestimated. Media such as those noted, to which malachite green had been added to a 1 : 1000 concentration, readily underwent putrefaction. These conclusions, however, simply refer to the dye itself and not to its compound with mercuric chloride as employed by Cheatle and his colleagues.

Malachite green belongs to the group of triphenylmethane dyes and may be represented by the following formula:



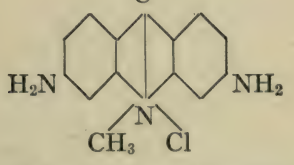
It may be prepared by a variety of methods, one of which, due to Doebner,[1] consists in heating dimethylaniline (2 mols.) with zinc chloride and benzotrichloride (1 mol.). The zinc salt obtained by this method is commonly converted into the oxalate which is the usual commercial form of malachite green. Browning has shown that some oxalates of dyestuffs, including malachite green and brilliant green, are more harmful to phagocytosis than other salts, so that it might be desirable to employ some soluble salt of malachite green other than the oxalate for antiseptic purposes.

Certain other members of the triphenylmethane group of dyes are known to possess definite bactericidal action, as shown by Dreyer, Kriegler, and Walker.[2] *Hexamethyl violet*,

[1] Liebigs Annalen, *217*, p. 250. [2] Journ. Path. and Bact., *15*, p. 133, 1910.

also known as "crystal violet," *hexaethyl violet*, and *brilliant green* are all credited with marked germicidal properties. The latter dye has been used to some extent by Browning and his surgical colleagues. Our own experiments do not indicate that brilliant green is nearly so powerful a germicide as claimed by Browning (cp. pp. 90, 95), although it doubtless may be of value in the treatment of certain types of wounds. A particularly exuberant growth of bright red granulation tissue is observed to follow its use. Brilliant green or tetraethyldiaminotriphenylcarbinol has a constitution similar to that of malachite green but with ethyl groups replacing the methyl groups of the latter. It is prepared like malachite green by heating diethylaniline with zinc chloride and benzotrichloride.

ACRIFLAVINE, TRYPAFLAVINE, OR FLAVINE. — This substance was first prepared by Benda[1] at Ehrlich's instigation in 1911 and was found to have a marked therapeutic effect on trypanosome infections. The systematic name for the compound is 3–6 diamino-10-methyl-acridinium chloride and is represented by the following formula:



The preparation of the compound was protected by patents and the registered trade mark "trypaflavine" was assigned to it. In order to avoid this name Browning and his colleagues used the simple term "flavine," but as a vegetable yellow dye of the same name has long been known this designation was

[1] Ber. deutsch. Chem. Gesell., *45*, p. 1787, 1912.

unfortunate. To avoid these and other important technical difficulties the Medical Research Committee has recommended[1] that so far as Britain is concerned the substance should be officially known as "acriflavine" and under this name various firms have obtained licenses to manufacture it.[2] In this way monopoly from the exploitation of the substance under fancy names has been obviated.

Acriflavine has been claimed by Browning and his associates[3] to be a most powerful antiseptic and it has found application in the prophylactic treatment of fresh wounds as well as in cases where suppuration has developed. It has also been employed in the disinfection of the nasopharynx of carriers of the meningococcus. Our own experiments on the germicidal action of acriflavine lead us to regard it as distinctly more active under most conditions than either malachite green or brilliant green (p. 97), though its rate of disinfection is decidedly slow. Direct observations on the bacterial count of infected wounds treated with 1 : 1000 acriflavine confirm our belief that the germicidal action of the substance has been overestimated.

One of the most remarkable properties of acriflavine is that its germicidal action is apparently enhanced by admixture with serum, though greatly diminished by pus. Relative to its bactericidal power, the dye is less detrimental to phagocytosis than most other antiseptics and it has but little injurious action on the tissues, but on the other hand its germicidal action is exerted decidedly more slowly than that of some commoner antiseptics. Its solutions may be boiled and can even be heated to 120° in the autoclave. It is generally used in 1 : 1000 solution in 0.8 per cent salt solution and may be employed for swabbing or syringing septic wounds once or twice daily according to the acuteness

[1] Brit. Med. Journ., June 9, 1917, p. 769.

[2] Acriflavine is, we believe, being manufactured by Messrs. Boot, Island St., Nottingham, and doubtless by other firms.

[3] Brit. Med. Journ., Jan. 20, 1917, p. 73.

of the condition. Gauze soaked in the solution may be placed next the wound and a protective covering put over the whole to hinder evaporation. Several ounces of 1 : 1000 acriflavine solution may safely be left in the tissues or peritoneal cavity. It may also be injected with a serum syringe into inflammatory areas. The opinion has been expressed that the special uses of acriflavine for particular purposes have still to be defined in relation to other antiseptics and to the operative methods of surgery, to which it can be at best only a valuable aid.

The following table, taken from Browning's paper, contains the results of bacteriological tests made with the various dyes mentioned. In judging of these results it must be borne in mind that relatively very small numbers of bacteria were employed (0.1 cc. of a 1 : 20,000 dilution in saline of a 24-hour peptone water culture) and that the action of the antiseptics was allowed to continue for 24 to 48 hours before examination.[1] A loopful of the mixture before adding the acriflavine is stated to yield twenty or more colonies. Such a low concentration of organisms in an old wound would indicate approaching surgical sterility, and moreover a survival of any number less than 5 per cent

[1] In the original paper by Browning and his colleagues certain antiseptics of the chlorine group, *e.g.* chloramine-T, eusol, chlorine water, and Dakin's solution, are included and an attempt is made to determine what is termed "antiseptic potency" as expressed by the ratio:

$$\frac{\text{Lethal concentration in serum of the substance in question}}{\text{Lethal concentration in serum of chloramine-T}}$$

These experiments are valueless since the chlorine antiseptics were added first to media, either peptone, water, or serum, which in the dilutions employed promptly decomposed most of them. For such experiments it is essential to add the disinfectant last, as in practical use, and there is little value in continuing experiments with rapid-acting unstable chlorine antiseptics for more than a few hours. The experiments as described give a false impression of the relative potency of acriflavine and similar dyes. The original statements by Dakin, Cohen, Kenyon, and Daufresne as to the germicidal action of hypochlorites and chloramine-T in water and serum have been repeatedly confirmed by ourselves and many others.

of the organisms in the test would have a good chance of being overlooked. As already stated, our own tests with heavily infected mixtures indicate much feebler germicidal action than that shown in the following table.

Antiseptic	Staphylococcus Aureus Lethal Concentration		Bacillus Coli Communis, Lethal Concentration		Concentration Which Inhibits Phagocytosis
	In Peptone Water 0.7 %	In Serum	In Peptone Water 0.7 %	In Serum	
Malachite green (oxalate and sulphate)	1 : 10,000,000	1 : 40,000	1 : 20,000	1 : 1000	1 : 7000
Brilliant green (sulphate)	1 : 10,000,000	1 : 30,000	1 : 130,000	1 : 3500	1 : 2000
Crystal violet	1 : 4,000,000	1 : 400,000	1 : 8000	1 : 8000	1 : 7000
Acriflavine	1 : 20,000	1 : 200,000	1 : 1300	1 : 100,000	1 : 500

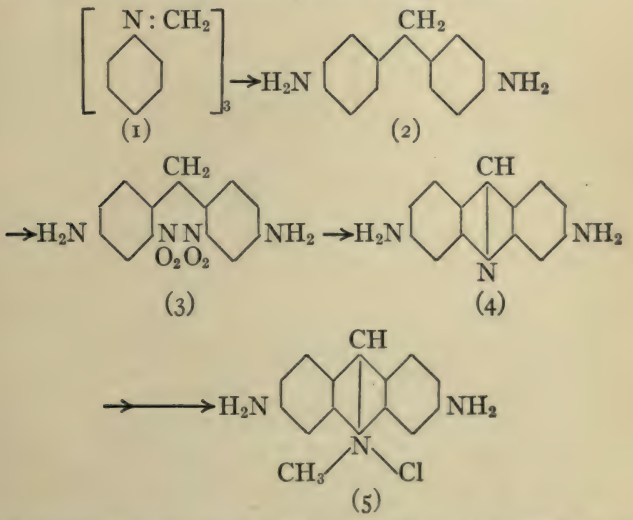
The preparation of acriflavine on a moderately large scale requires a considerable degree of chemical skill. Several methods for the synthesis of acriflavine and related acridine derivatives [1] are known but only one of them need concern us here. The working details must be sought in the original communication.[2]

Aniline and formaldehyde unite to give a polymeric substance, anhydroformaldehyde-aniline (1). When heated with aniline hydrochloride it undergoes a curious rearrangement with the formation of p-diaminophenylmethane (2). This substance is nitrated with nitric and sulphuric acids so as to give a dinitro derivative (3); which in turn is reduced with

[1] Bucherer's Chemie der Tierfarbstoffe, 1914, may be consulted for information concerning the synthesis of acridine derivatives.

[2] Benda, Ber. deutsch. Chem. Gesel., *45*, p. 1787, 1912; D. R. Patents, Kl. 22b, 230412 and 243085, Casella and Co., 1911–1912.

tin and hydrochloric acid. On heating the reaction mixture in an autoclave to 135°, 3-6-diamino acridine is obtained (4). In order to obtain the methyl derivative the latter compound is first converted into its di-acetyl compound to protect the amino-groups and then treated in nitrobenzene solution at 175° with the methyl ester of toluene sulphonic acid. The product is then hydrolyzed, when acriflavine (hydrochloride) = 3-6-diamino-10-methylacridine (5) results:



Apparently the germicidal properties of acriflavine are shared by a number of other acridine derivatives and one of these, diaminoacridine, (4) is easier and cheaper to manufacture and appears to be equally desirable. It will be known as "proflavine." A detailed publication on this substance is expected shortly. Experiments on its germicidal action are included in Chapter VII.

CHAPTER VI

MISCELLANEOUS ANTISEPTICS

In this section brief reference will be made to a few antiseptics which cannot be included in preceding sections. It will be impossible to treat these substances systematically, and instead brief notes as to the chief characteristics of the substances is all that will be attempted.

Hydrogen Peroxide and other Peroxides. — Hydrogen peroxide is not held in very high repute as a germicide but it has certain other qualities which render it decidedly valuable. When tested against relatively small quantities of *staphylococcus aureus* or *B. pyocyaneus* in water, a concentration of 0.03 per cent actual H_2O_2, *i.e.* a 1 per cent dilution of the pharmacopœal product, may suffice to sterilize in two hours, while in blood serum about double this concentration may be necessary. But these conditions are entirely artificial and unrelated to what happens when hydrogen peroxide is applied to a septic wound. Blood, pus, and muscle juice contain an enzyme "catalase" which rapidly brings about the decomposition of hydrogen peroxide with liberation of gaseous oxygen. This rapid decomposition with evolution of gas soon decomposes all the peroxide and its disinfecting action comes to a speedy end. The mechanical effect of the disengagement of gas is often a valuable property and is made use of in loosening sticky secretions, washing away pus, or loosening adherent dressings. For such purpose it is of great value but it is important that its transient germicidal effect should be recognized.

A great variety of other peroxides have been put forward as disinfectants, but most of them are of doubtful practical value. Two of the most active of these substances are the benzoyl hydrogen peroxide and benzoyl acetyl peroxide discovered by Baeyer and studied by Freer and Novy. The latter compound has been used to some extent but the unstable character of both the substance and its solutions has prevented its extensive employment. Dibenzoyl peroxide has been recommended as an antiseptic but it is a sparingly soluble, practically indifferent compound of no significant germicidal value.

Ozone. — This substance has recently been used by Stoker[1] for the treatment of infected wounds, especially cavities and sinuses in bone injuries. An Andreoli ozonizer is used to produce the ozonized air which is allowed to act for about fifteen minutes or such shorter period as produces a superficial glazing of the wound surfaces. Details of ozone concentration and bacteriological controls are not yet available, although the results are stated to be good and the formation of excessive granulation tissue is avoided.

Iodine. — Extensive use is made of iodine as a germicide. Its action is powerful and prompt when the conditions are such that the antiseptic has free excess to the microörganisms. Ampoules containing tincture of iodine have been used largely in the present war as a first aid treatment to be applied by the wounded soldier. In general the results as regards the prevention of sepsis have been disappointing, mainly it would appear because the injured man is not apt in most cases to put the iodine where it can reach the focus of infection. The presence of much blood and prolonged oozing are of course inimical to the exercise of germicidal action. Usually little more is accomplished than a fair cleansing of the adjacent skin.

[1] Lancet, Oct. 21, p. 712, 1916.

The use of iodine as a skin disinfectant introduced by Stretton[1] in 1909 is widely practiced and undoubtedly it has great value for this purpose. A 2.5 per cent solution is usually strong enough and alcohol is generally employed as the solvent. Seventy per cent alcohol is preferable to stronger spirit and it is important to use pure alcohol as otherwise iodoacetone and other products are apt to be formed, which are very irritating to the eyes of the operator.

Light petroleum and heavy mineral oils are also used as solvents for iodine and act well. They have the advantage of being stable and cheap. Dichlorethylene (*i.e.* acetylene dichloride) has also been proposed but is much more expensive.

The use of iodine for the antiseptic treatment of large war wounds is now practiced much less frequently than before, although it was given a thorough trial in the early days of the war. It has been found much too irritating for repeated application and not infrequently objectionable after effects such as severe neuritis have been observed. Its strong coagulating action on proteins is also an objectionable feature. In general it may be said that iodine will be found most useful when the conditions are such that rapid and complete sterilization may be effected by a single application as in skin disinfection or small surface wounds.

An experiment illustrating the rapid effect of 2 per cent iodine solution on staphylococci and other organisms suspended in a blood serum-muscle extract medium will be found on p. 86. A 1 per cent solution added to an equal volume of blood heavily infected with streptococci did not kill all the organisms in one hour according to Emery. Against staphylococci suspended in water, about ten million per cubic centimeter, 1 : 100,000 iodine is effective in two hours, while in blood serum 1 : 1000 is required, according to our own observations.

[1] Brit. Med. Journ., Aug. 14, 1909, May 22, 1915.

BORATES, PERBORATES, AND BORIC ACID. — These substances while possessing almost negligible germicidal properties find extensive use when a bland, mildly antiseptic lotion is required. Sodium monoborate is a rather strongly alkaline salt but is said to be non-irritating to wounds; borax, or sodium biborate is less strongly alkaline, while boric acid is feebly acid. All of these substances are used for restraining the growth of putrefactive organisms rather than for disinfection in the true sense. Sodium perborate is prepared by adding hydrogen peroxide to borax solutions and is stable when preserved in the dry state. It is often used as a convenient substitute for hydrogen peroxide.

PERSULPHATES. — The use of potassium or sodium persulphate has been advocated but actually their disinfecting action on pyogenic organisms is feeble. A solution of the sodium salt has been regarded by some as useful for stimulating the rate of cicatrization of wounds, though this action can hardly be regarded as satisfactorily demonstrated.

ACIDS. — Almost all acids possess some germicidal action and generally speaking their activity is proportional to their "strength." Just as metallic "ions" appear to be the actual disinfecting agent when metallic salts are employed, so in the case of most acids it is the hydrogen ions which seem to be effective. Extensive experiments on this subject have been carried out by Bial[1] and by Winslow and Lockridge.[2] A few experiments on the action of various acid substances on *B. typhosus* will be found on p. 114.

Occasionally acids have been employed in wound treatment and it has been thought that irrigation with weak lactic acid was useful in inhibiting the growth of the gas bacillus in infected wounds, but the treatment has not met with general favor.

[1] Archiv. exper. Path. a. Pharm., *38*, p. 1, 1897. Zeitschr. f. physiol. Chem., *40*, p. 513, 1902.

[2] Journ. of Infectious Diseases, *3*, p. 547, 1906.

Alcohol and Ether. — Both of these substances are employed in the treatment of infected wounds but they are used as much for the help they afford in the mechanical cleaning of dirty wounds as for any direct germicidal action. Most vegetative forms of bacteria may be killed fairly readily by 50 per cent alcohol but alcohol of much lower or higher strength is less effective, while most spores are unaffected by alcohol of any strength. The following table contains the results of experiments by Minervini [1] to determine the time required to kill various organisms in alcohol of varying concentration. Koch's "thread" method of testing the viability of the organisms was used and the results are at least comparable among themselves.

Organism	Dilution of Alcohol				
	25 %	50 %	70 %	80 %	99 %
Staphylococcus aureus	12–24 hours	10 min.	10 min.	living after	3 days
B. pyocyaneus . . .	under 1 hour	10 min.	10 min.	6 hours	12 hours
M. prodigiosus . .	1 hour	10 min.	10 min.	6 hours	12–24 hours
B. coli	24 hours	1 hour	1 hour	living after	24 hours
B. subtilis (spores) .	all living after 8 days				
B. anthracis (spores)	all living after 50 days				

It is well known that alcohol or glycerol materially reduces the germicidal efficiency of some antiseptics, particularly those of the phenol class. Krönig and Paul found that phenol dissolved in 98 per cent alcohol was devoid of bactericidal action when tested against spores. Cooper [2] has correlated this fact with a diminished protein precipitating action of alcoholic phenol when contrasted with aqueous phenol, but whatever the explanation may be it is clear that alcohol is not a desirable solvent for phenolic disinfectants.

[1] Cp., Rideal, Disinfection and Disinfectants, p. 322.
[2] Biochem. Journ., 7, p. 175, 1913.

The germicidal properties of ether have recently been examined by Topley,[1] who finds that the vapors possess a slight but definite action. An exposure to ether vapors of one to forty-eight hours was necessary to sterilize agar slants on which pyogenic organisms were growing. Liquid ether was irregular in its action and good contact with the organisms was difficult to secure. It is clear, however, from these and from clinical results that any beneficial effects following the use of ether in the treatment of septic infections is not due to direct disinfection.[2]

FORMALDEHYDE. — The physical and chemical properties of formaldehyde, which is obtainable in commerce as a 40 per cent solution known as "formalin," make it valuable for various forms of disinfection, such as the fumigation of rooms, etc. But so far as the treatment of septic wounds is concerned it has not proved particularly successful and is now scarcely used for such purposes. It is employed to some extent for the sterilization of the hands and instruments but it is less popular than formerly. A 0.5 per cent solution is generally employed for such purposes. It is not a very rapid acting disinfectant and when tested in the ordinary way under the standard conditions it has a phenol coefficient of about 0.4, but if the time of action is prolonged its activity may be somewhat greater than that of phenol.

HEXAMETHYLENETETRAMINE. — This substance, obtained by the action of ammonia on formaldehyde, has but little direct germicidal action. In acid solution it may be decomposed with liberation of formaldehyde which can thus exert its antiseptic action. A large number of derivatives of hexamethylenetetramine have been prepared by Jacobs and Heidelberger and many of these are more powerful than the parent substance, but their useful application has still to be defined. Hexamethylenetetramine itself is not suit-

[1] Brit. Med. Journ., Feb. 6, 1915.
[2] Cp. Distaso and Bowen, Brit. Med. Journ., Feb. 24, 1917.

able for wound treatment since under these conditions its germicidal action is quite inadequate.

IODOFORM. — The use of iodoform in combination with bismuth subnitrate and paraffin oil in the mixture known as "B. I. P." has already been referred to on p. 58. The substance was once considered to be a powerful antiseptic but this is no longer believed to be the case. Microörganisms may flourish in contact with iodoform but, on the other hand, it is not disproved that in contact with living tissues iodoform may be slowly decomposed with the formation of products of genuine antiseptic value. Apart from the paste referred to, its use in surgery is diminishing, probably on account of occasional unpleasant poisoning effects that may follow its free use, especially in confined cavities. Many odorless substitutes for iodoform are known under various trade names, but in general their action appears to be similar to that of iodoform itself.

PERMANGANATES. — The potassium salt is principally used for irrigation, 1 : 1000, in gonorrhœa. It is an active germicide under conditions not involving rapid decomposition by excess of organic matter. All the permanganates are strong oxidizing agents and as soon as they are reduced to manganese salts their disinfecting action ceases, so that their maximum germicidal effects are transitory. They are unsuitable for septic wound treatment on account of their rapid reduction but have many other useful applications.

QUININE. — The hydrochloride of this alkaloid has been recommended by Kenneth Taylor [1] as a dressing for septic wounds, especially those infected with the *B. ærogenes capsulatus*. The substance is used in 0.1 per cent aqueous solution with the addition of 0.1 per cent hydrochloric acid or 1 per cent alcohol to inhibit precipitation of the base. Tested *in vitro* Taylor finds that it is about ten times as effective as phenol against the gas bacillus although, on the

[1] Lancet, Sept. 4, 1915. Brit. Med. Journ., Dec. 25, 1915.

other hand, it is used on wounds in much lower concentration than is usual with phenol. Serum or pus is stated to have no very marked action in reducing the germicidal activity of quinine. The solution is used either as a wet dressing or for continuous drip instillation. A curious fact noted by Taylor in connection with the use of quinine is that while there appears to be a decrease in *B. ærogenes capsulatus* infection there was a progressive increase in the appearance of *B. pyocyaneus*, and laboratory tests showed that the latter organism was the most resistant to quinine of the common pyogenic bacteria.

An endeavor has been made to utilize the combined anæsthetic and antiseptic properties of mixtures of quinine and urea for wound treatment, but this has failed to secure much favor.

Chinosol. — This compound was originally considered to be potassium oxyquinoline sulphonate or a double salt of this with potassium sulphate, but is now stated to be neutral oxyquinoline sulphate. It possesses a strong inhibitory action on the growth of many microörganisms and failure to recognize this fact fully led to exaggerated claims as to its germicidal potency. A full report upon this substance has been made by the Council on Pharmacy and Chemistry of the American Medical Association.[1] The general conclusions arrived at are essentially as follows: As regards *staphylococcus aureus* and *B. typhosus* chinosol is more strongly *antiseptic* than phenol and about equal to mercuric chloride, but as a *germicide* in watery solution it is somewhat inferior to phenol and vastly inferior to mercuric chloride. In acid broth the findings were still less favorable to chinosol.

These conclusions make it doubtful if much direct germicidal effect on wounds can follow its application, though it is possible that the substance might find useful employment

[1] Cp. Journ., Am. Med. Assoc., May 28, 1910.

as a wound dressing with the object of restraining the growth of organisms and preventing reinfection. It does not appear to have been used extensively in the present war.

Acetanilide and its Derivatives. — This substance has often been recommended as an antiseptic,[1] but our own experiments have shown its germicidal properties when tested against moderate quantities of staphylococci to be so low that it can hardly exert any effective disinfection in wounds. A half per cent solution failed to sterilize staphylococci in water in two hours, while in blood serum its antiseptic action is negligible. Recently E. F. Greene[2] has revived the question of its possible use in infected war wounds, applied as a dry powder, but this can hardly be endorsed, not only on account of its poor antiseptic action but also because symptoms of poisoning have been stated to follow its use. Its free application to extensive wounds would certainly not be without danger to many individuals who are fairly susceptible to this drug.

Various derivatives of acetanilide in which the hydrogen of the benzene ring has been replaced by chlorine, bromine, or iodine have been advocated as antiseptics from time to time. Parabromacetanilide is sometimes known under the name of "asepsin" or "antisepsin" and is stated to have anodyne properties. Our own experience indicates that none of these derivatives are sufficiently active disinfectants to be of value in wound treatment.

[1] Cp. Beck, New York, Med. Journ., March 19, 1893.
[2] Brit. Med. Journ., May 29, 1915, p. 928.

CHAPTER VII

METHODS OF TESTING ANTISEPTICS

THE testing of substances for their antiseptic and germicidal power is fraught with innumerable pitfalls. It is possible to take almost any substance and by carefully choosing the conditions under which it acts, make it appear to possess germicidal potency, and conversely, it is equally possible to take valuable germicides and by observing their action under unreasonable conditions, make them appear inert. The necessity for choosing methods of testing in some degree in conformity with the mode of use of the substance and under conditions not too remote from those under which it is proposed to employ it, is of fundamental importance.

The simplest conditions are presented in the determination of the lethal concentration of a germicide acting upon microorganisms suspended in water. The chief variables in such a determination are the number of organisms taken for each experiment, and the temperature and time of action. With regard to the number of organisms, it is desirable to use a fairly large quantity, partly because spontaneous death of the bacteria then becomes less of a factor and also because the test becomes a more rigid one. In general, some such concentration as a hundred million bacteria to the cubic centimeter will be found appropriate. The effect of temperature on the rate of disinfection has already been considered, also the speed of action will be found to vary enormously with different substances and with varying media.

One of the oldest methods used for the determination of lethal concentrations is the so-called "thread" method devised by Koch. Sterile raw silk thread cut into portions about a centimeter long are soaked in a broth culture or aqueous suspension of the required organisms and then dried. These threads are soaked for an arbitrary time in varying concentrations of the antiseptic, then removed and transferred to sterile broth, which on incubation will show whether or not the organisms were killed. One of the main objections to this method is the mechanical carrying over of adhering antiseptic into the broth medium, so that inhibition of growth frequently occurs even though the organisms are not killed, and the substance appears to be a more effective germicide than is actually the case. Failure of the antiseptic to promptly penetrate to all parts of the thread may lead to erroneous estimates of its germicidal power.

A later method, which is often called the "garnet" method, was worked out by Krönig and Paul. Garnets of equal size are dipped in an emulsion of the organism — usually spore-bearing anthrax bacilli — and then carefully dried so that a thin film of organisms is spread over their surface. The garnets are then immersed in a solution of the antiseptic, which after a definite time is gently washed away and the garnets, if need be, treated with an antidote such as ammonium sulphide when mercury disinfectants are employed. The garnets are then shaken with water to detach a fairly constant proportion of the organisms and an aliquot part of the fluid is plated in solid media for counting. An alternative method using the surface of nutrient agar instead of garnets or thread, is described by Bechold and Ehrlich.[1]

But neither the "thread" nor "garnet" method is often used now and the simpler method is usually employed of mixing known but varying quantities of the disinfectant with a constant concentration of organisms and subculturing from

[1] Zeit. physiol. Chem., *47*, p. 177, 1906.

the mixture into broth or agar at known intervals of time, in order to determine whether disinfection has been completed.

An application of this method, which is of great value in standardizing and comparing different substances with regard to their practical uses as disinfectants in hygienic work, is that devised by Walker and Rideal[1] in 1903. By this procedure it is possible to express the value of a disinfectant in terms of a numerical ratio using pure phenol as a standard. The original method is substantially as follows: a definite amount (usually 5 drops) of a 24-hour broth culture of the organism selected, *e.g. B. typhosus* or *B. coli*, is added to 5 cc. of solutions of varying concentration of the disinfectant. Subcultures are taken at intervals of $2\frac{1}{2}$ minutes up to 15 minutes to determine the point of complete sterility. A similar series of tests is made with a standard phenol solution of such strength that sterilization of the organisms will be effected within the time limits of $2\frac{1}{2}$ to 15 minutes. The necessary concentration of phenol will usually be about 0.8 per cent with most cultures of *B. typhosus*. By comparing the concentration of the disinfectant which just effects sterilization in a given time with the phenol concentration which also effects sterilization in the same time, an estimate is formed of the germicidal potency of the substance under investigation. Thus if a concentration of 1 : 600 of a particular substance is as effective under the above conditions and in the same time as 1 : 120 phenol, the "phenol coefficient" will be $\frac{600}{120}$ or 5. It is an essential and important feature of this method that time of action should be constant while the concentration of the disinfectant is varied. The reverse conditions, namely, fixed concentration and variable time, may lead to entirely erroneous inferences as to germicidal potency.

[1] Journ. Roy. Soc. Inst. *24*, p. 424, 1903. Later details concerning the Walker-Rideal method will be found in The Amer. Journ. Pub. Health, Vol. 3, No. 6.

The determination of the "phenol coefficient" of a substance furnishes most valuable information, but chiefly as regards the relative value of disinfectants to be used in fairly homogeneous aqueous media. Chick and Martin[1] and many others cited in the paper by these authors have sought to obtain a more practical estimate of the germicidal value of disinfectants by adding organic matter of various kinds to the bacterial suspension. For some types of general disinfectants the addition of a definite quantity of dried feces is recommended, for example, when testing those substances destined to be used for the disinfection of stools.

The problem of selecting conditions for studying the action of substances to be used as wound disinfectants is much more difficult. Useful information is obtained by determining the lethal concentration of substances acting on organisms suspended in blood serum or in blood, or by studying their action on pus. Certain precautions must be taken in working with these media. With all of them, it is of course essential that, as in practical use, the antiseptic solution should be added to the mixtures last, for in many cases if this is not done totally erroneous results will be obtained. It has happened with extraordinary frequency that small quantities of labile chlorine antiseptics have been added to organic media and then at unstated intervals after all or most of the antiseptic has been destroyed, the mixture is infected with organisms which naturally grow unchecked. Under such conditions, what is really being observed is less the germicidal action of the antiseptic than the rate of chemical reaction which has taken place between the unstable antiseptic and organic media. The results of such a procedure are naturally particularly misleading when high dilutions of antiseptic are employed in experiments to determine the lethal concentration.

[1] Journ. Hygiene, *8*, p. 654, 1908.

A great advantage in the use of serum is the fact that it presents a homogeneous medium of fairly constant composition; but it is important to bear in mind that many organisms, *e.g. B. paratyphosus*, are very susceptible to the action of blood serum even though previously heated to 58–60° for an hour. To avoid this complication, liberal quantities of an organism such as *staphylococcus aureus*, which is fairly resistant to serum, should be used. When substances of high germicidal power are examined for the determination of the lethal concentration, and hence low concentrations are employed, it will usually be found sufficient to carry out the tests in a medium containing 50 per cent or even less of serum, since its mass, relative to that of the antiseptic, will be very large.

The testing of the germicidal effects of substances acting on organisms suspended in blood would seem the rational procedure for examining compounds which might be used intravenously. A blood medium has, however, been suggested as a standard method of testing ordinary antiseptics by Emery.[1] The use of a blood medium as a standard is particularly adverse to the hypochlorite antiseptics which rapidly react with hæmoglobin so that a relatively considerable amount of the former must be added before any active antiseptic will persist in the mixture. For these tests use may be made of reconstituted blood obtained by mixing serum with cells separated from citrated blood, although for most purposes defibrinated blood or citrated blood itself would probably serve. The technique employed by Emery is substantially the following: Nine parts of "reconstituted blood" is mixed with one part of a streptococcus culture containing about 250,000,000 organisms per cubic centimeter. One volume of the infected blood is mixed on a slide, using a marked capillary pipette, with an equal volume of the antiseptic solution. The mixture is then drawn

[1] Lancet, April 15, 1916, p. 817.

into the pipette, which is sealed and incubated and subsequently examined to determine whether the contents are sterile or whether viable organisms persist. It should be noted that apparently the concentrations of antiseptic quoted in Emery's table refer to the strength of the solution added, so that their actual concentration in the final mixture is half of that given.

Antiseptic	15 Minutes		60 Minutes	
	Did not kill	Killed	Did not kill	Killed
Phenol	1:70	1:60	1:60	1:50
Eusol (HClO)	[1:400?]	—	—	—
Sodium Hypochlorite	[1:200?]	—	—	—
Mercuric Chloride	1:100	1:80	1:100	1:80
Mercury Biniodide	1:60	1:40	1:60	1:40
Iodine	1:100	?	1:100	?
Lysol	1:40	1:30	1:150	1:120
Malachite Green	1:250	1:200	1:250	1:200

The results recorded by Emery do not make the method as described by him appear particularly accurate, since with only one exception as high a concentration was necessary to sterilize the mixture in 60 min. as in 15 min., although the act of disinfection is a progressive time reaction. Moreover, a higher concentration of phenol is recorded as necessary to sterilize in sixty minutes than is stated to be effective in fifteen. But the use of blood as a medium for studying the germicidal action of substances in the presence of cellular elements, is of distinct value since, unlike pus, its composition is fairly uniform. The blood should be heavily infected with organisms so that its natural bactericidal properties will not effect the end result materially. On pp. 93, 95 a number of experiments with antiseptics of the chlorine group and dyestuffs, acting on staphylococci in blood media, are recorded.

The determination of the disinfecting action of substances upon pus is obviously important as bearing on the treatment of suppurating wounds. As already stated, the disinfection of pus is difficult of accomplishment and relatively high concentrations of antiseptic are necessary. But great difficulties are encountered in laboratory experiments on the disinfection of pus owing to the enormous variations in its physical condition and the number of organisms it contains and whether these organisms are mainly free or ingested in the leucocytes. Rous and Jones [1] have shown that living phagocytes are able to protect ingested organisms from the action of substances such as potassium cyanide in the surrounding fluid and even from the action of a strong homologous antiserum, and they obtained evidence that this protection by phagocytes was largely conditioned on their being alive. But even when all the leucocytes are dead, there are considerable mechanical difficulties in securing good contact with the antiseptic solution. An interesting series of experiments on the sterilization of pus by various antiseptics is recorded in a paper by Parry Morgan,[2] and some of the results have already been cited on p. 8.

Thus far, we have considered almost exclusively the question of the lethal concentration of disinfectants acting under different conditions, but it is becoming constantly more evident that much more than this is needed in judging of the utility of antiseptics. As already stated on p. 6, the act of disinfection resembles in many respects an ordinary chemical reaction in which the two reacting components are represented by disinfectant and bacterial protoplasm. Now the rate of disinfection varies enormously with different germicides, and is of course influenced by the relative mass of bacteria and disinfectant, as well as by temperature and contact between the reacting substances. The instability

[1] Journ., Exp. Med., *23*, p. 601, 1916.
[2] B. M. J., May 13, 1916, p. 684.

of many antiseptics, leading to a reduction or disappearance of the mass of active antiseptic, must also be reckoned with. A knowledge of the speed with which a disinfectant acts is essential to an understanding of the conditions under which it may be appropriately used. For example, the extremely rapid acting aqueous hypochlorites are admirably adapted for intermittent instillation in large quantities into wound cavities, while when the same solutions are applied as wet dressings which are seldom renewed, their action is over as soon as the active chlorine has disappeared, and this may be a matter of only seconds or minutes. For prolonged action when intermittent instillation cannot be practiced, a slower acting but more stable antiseptic is likely to give better results, or they may be attained by using an oil solution of dichloramine-T (p. 34) from which the active antiseptic slowly passes from the oil to the aqueous medium on the surface of the wound.

The only satisfactory way to follow the speed of disinfection is to determine the progressive change in the number of bacteria in a suitable mixture after varying lengths of time. With this end in view, we have made a number of time experiments with various antiseptics, using them in most cases at about the concentration which is recommended for wound treatment. The mixtures contained 1 cc. of horse blood serum, 1 cc. of muscle extract obtained by soaking fresh veal with an equal weight of saline and then straining through cloth but not filtering, and 2 drops of a *staphylococcus aureus* emulsion obtained by shaking a 24-hour agar slant of abundant growth with 8 cc. of saline. The muscle extract doubtless contained organisms of other kinds in fair quantity. After determining the total number of organisms present in the mixture by plating an aliquot part, 1 cc. of antiseptic solution was added and samples withdrawn from time to time and the surviving bacteria estimated. In the case of chlorine antiseptics, their further

action after sampling was checked by sodium thiosulphate, while potassium sulphide was used as an antidote with the salts of the heavy metals. No antidote was used with the dyes. The use of the blood serum-muscle extract medium was chosen as bearing some similarity to the composition of wound exudate and it has the further advantage of being easily reproduced in fairly uniform quality. For many antiseptics, the addition of muscle extract makes the tests much more severe than when blood serum alone is used. The experiments were all carried out at 32–35° C. and the results are expressed as the number of bacteria present in one standard drop of the mixture = 1/40 cc.

I. Chlorine Group of Antiseptics

(Blood Serum-Muscle Extract Medium; Staphylococcus aureus.)

Antiseptic Used	Concentration		Time of Action	Bacterial Count 1 Drop = 1/40 cc.
	As Added	In Mixture		
I. Sodium Hypochlorite .	0.5%	0.17%	0	1 966 000
			2 min.	405
			5 min.	0 [1]
II. do. do. . .	0.5% (0.2 cc.)	0.05%	0	1 966 000
			5 min.	311 200
			15 min.	157 400
			45 min.	98 170
			1.5 hr.	16 120
			3 hr.	1 651
			6 hr.	1 587
			24 hr.	1 294 000 [2]
III. Eusol	0.27%	0.09%	0	2 150 000
			2 min.	496
			5 min.	2
			15 min.	0 [1]

[1] Active chlorine present.

[2] Active chlorine absent.

I. CHLORINE GROUP OF ANTISEPTICS — *Continued*

(Blood Serum-Muscle Extract Medium; Staphylococcus aureus.)

ANTISEPTIC USED	CONCENTRATION		TIME OF ACTION	BACTERIAL COUNT 1 DROP = $\frac{1}{10}$ CC.
	As Added	In Mixture		
IV. Eusol	0.27% (0.2 cc.)	0.025%	0	2 129 000
			5 min.	1 310 000
			15 min.	507 900
			45 min.	606 200
			1.5 hr.	641 400
			3 hr.	868 300
			6 hr.	983 000
			24 hr.	2 310 000 [2]
V. Chloramine-T	2%	0.67%	0	1 365 000
			5 min.	0 [1]
VI. do.	0.5%	0.17%	0	726 300
			5 min.	998
			15 min.	191
			45 min.	7
			1.5 hr.	4
			3 hr.	3
			6 hr.	0 [2]
VII. Dichloramine-T in Oil (v, p. 39)	2%	0.67% [3]	0	2 020 000
			0.5 min.	0
VIII. do.	2%	0.67% [4]	0	1 157 000
			5 min.	458 700
			15 min.	294 900
			45 min.	0
IX. Iodine in Potassium Iodide	2%	0.67%	0	1 463 000
			5 min.	0

The results shown in Table I in which members of the chlorine group of antiseptics and iodine are considered, il-

[1] Active chlorine present.
[2] Active chlorine absent.
[3] Mixed with platinum wire.
[4] Not mixed, left to diffuse.

lustrate their extraordinary speed and completeness of disinfection when added in adequate amount to the infected mixture. In Experiments I, III, V, VII, and IX, $\frac{1}{8}$ volume of the various antiseptic solutions was added at a concentration no higher and in some cases lower than that commonly employed in wound treatment and in every case practical sterility was obtained in less than five minutes. When much smaller amounts of hypochlorites were added, as in Experiments II and IV, their rapid action is seen to cease as soon as all the active antiseptic is decomposed and subsequent growth can then take place. In Experiment VI, in which weak (0.5 per cent) chloramine-T was used, its action is shown to be distinctly more effective than an approximately equivalent amount of chlorine in the form of sodium hypochlorite or hypochlorous acid (Experiments II and IV).

II. Metallic Salts, Phenol, Hydrogen Peroxide

(Blood Serum-Muscle Extract Medium; Staphylococcus aureus.)

Antiseptic Used	Concentration		Time of Action	Bacterial Count 1 Drop = $\frac{1}{10}$ cc.
	As Added	In Mixture		
I. Mercuric Chloride . .	0.1%	0.033%	0	1 894 000
			5 min.	254 400
			15 min.	44 920
			45 min.	12 900
			1.5 hr.	7 258
			3 hr.	2 985
			24 hr.	0
II. Silver Nitrate . . .	1%	0.33%	0	786 400
			5 min.	720 900
			15 min.	651 700
			45 min.	530 600
			1.5 hr.	425 900
			3 hr.	175 600
			6 hr.	38 270
			24 hr.	2 643

II. METALLIC SALTS, PHENOL, HYDROGEN PEROXIDE — *Continued*

(Blood Serum-Muscle Extract Medium; Staphylococcus aureus.)

ANTISEPTIC USED	CONCENTRATION		TIME OF ACTION	BACTERIAL COUNT 1 DROP = $\frac{1}{60}$ CC.
	As Added	In Mixture		
III. Argyrol	15%	5%	0	917 500
			5 min.	753 500
			15 min.	655 300
			45 min.	622 500
			1.5 hr.	327 600
			3 hr.	9 792
			6 hr.	4 693
			24 hr.	0
IV. Zinc Chloride	3%	1%	0	1 223 000
			5 min.	868 300
			15 min.	819 200
			45 min.	491 500
			1.5 hr.	211 900
			3 hr.	51 580
			6 hr.	3 667
			24 hr.	704
V. Hydrogen Peroxide .	2.89%	0.96%	0	1 201 000
			5 min.	589 800
			15 min.	819 200
			45 min.	778 200
			1.5 hr.	802 800
			3 hr.	1 087 000
			6 hr.	1 136 000
VI. Phenol	2%	0.67%	0	1 409 000
			5 min.	1 471 000
			15 min.	819 200
			45 min.	192 700
			1.5 hr.	34 900
			3 hr.	13 700
			24 hr.	2 985

In the second series of experiments a similar infected medium of blood serum and muscle extract was mixed with solutions of various metallic salts, phenol, and hydrogen

peroxide. Mercuric and zinc chlorides and silver nitrate produced immediate precipitates. The action of mercuric chloride 0.1 per cent is seen to be more rapid than that of phenol 2 per cent, silver nitrate 1 per cent, or zinc chloride 3 per cent. Argyrol was tried in very high concentration and while acting slowly sterilized completely in 24 hours. Neither phenol, silver nitrate, nor zinc chloride sterilized completely in 24 hours, although the number of viable organisms was less than 1 per cent of those originally present in the mixture. The effect of hydrogen peroxide is interesting as showing an extremely rapid but transitory action which reached its maximum in a few minutes, after which the organisms grew unchecked.

III. Dyes

(Blood Serum-Muscle Extract Medium; Staphylococcus aureus.)

Antiseptic Used	Concentration		Time of Action	Bacterial Count 1 Drop = $\frac{1}{10}$ cc.
	As Added	In Mixture		
I. Malachite Green . . (Grübler)	0.3%	0.1%	0	821 600
			5 min.	182 700
			15 min.	60 920
			1.5 hr.	8 920
			3 hr.	5 574
			6 hr.	78 200
			24 hr.	1 015 000
			48 hr.	3 706 000
II. do. . . do. . . .	0.1%	0.033%	0	2 097 000
			5 min.	1 359 000
			15 min.	369 100
			45 min.	211 500
			3 hr.	272 300
			6 hr.	557 000
			24 hr.	1 096 000
			48 hr.	6 553 000

III. DYES — *Continued*

(Blood Serum-Muscle Extract Medium; Staphylococcus aureus.)

ANTISEPTIC USED	CONCENTRATION		TIME OF ACTION	BACTERIAL COUNT 1 DROP = $\frac{1}{10}$ CC.
	As Added	In Mixture		
III. Brilliant Green . . . (Grübler)	0.3%	0.1%	0	810 200
			5 min.	29 950
			15 min.	27 410
			45 min.	20 160
			1.5 hr.	14 310
			3 hr.	8 871
			6 hr.	1 946
			24 hr.	256 600
			48 hr.	658 900
IV. Acriflavine	0.3%	0.1%	0	589 800
			5 min.	358 400
			15 min.	249 300
			45 min.	179 200
			1.5 hr.	78 210
			6 hr.	704
			24 hr.	0
V. do.	0.1%	0.033%	0	557 100
			5 min.	218 600
			15 min.	121 900
			45 min.	100 800
			1.5 hr.	81 540
			6 hr.	474
			24 hr.	0
VI. do.	0.03%	0.01%	0	755 400
			5 min.	524 300
			15 min.	319 300
			45 min.	308 200
			1.5 hr.	106 500
			3 hr.	94 840
			6 hr.	22 780
			24 hr.	1 113 000
VII. Proflavine	0.3%	0.1%	0	888 100
			5 min.	794 500
			15 min.	744 500
			45 min.	308 200
			1.5 hr.	104 800
			3 hr.	66 560
			6 hr.	53 250
			24 hr.	1 242

III. DYES — *Continued*

(Blood Serum-Muscle Extract Medium; Staphylococcus aureus.)

ANTISEPTIC USED	CONCENTRATION		TIME OF ACTION	BACTERIAL COUNT 1 DROP = $\frac{1}{10}$ CC.
	As Added	In Mixture		
VIII. Proflavine	0.1%	0.033%	0	888 100
			5 min.	744 500
			15 min.	655 400
			45 min.	326 100
			1.5 hr.	169 900
			3 hr.	154 800
			6 hr.	84 900
			24 hr.	4
IX. do.	0.03%	0.01%	0	855 400
			5 min.	761 800
			15 min.	744 500
			45 min.	469 500
			1.5 hr.	304 500
			3 hr.	196 400
			6 hr.	93 750
			24 hr.	183 600

In the third series of experiments the action of malachite green, brilliant green, and acriflavine acting in a similar mixture of blood serum and muscle extract were examined. The germicidal action of these dyes, in view of claims made for them, proved disappointing. Brilliant green has been recommended for use by Browning and his colleagues at a concentration of 0.1 per cent but addition of one third volume of brilliant green or malachite green at this concentration gave but poor evidence of much germicidal action. Subsequent experiments with these two dyes were made at a higher concentration, 0.3 per cent. In no case was sterility reached and after some hours the organisms grew unchecked. It was noted that similar mixtures of blood serum and muscle

extract mixed with these dyes so that the final concentration was 0.1 per cent, on exposure to air readily underwent thorough putrefaction. It would appear that under the conditions of these experiments the dyes in question are readily inactivated and that the presence of apparently unchanged coloring matter in the mixture is no evidence of the continuation of any antiseptic action. Similar experiments with acriflavine, 0.1 per cent, showed a slow initial effect but antiseptic action was more sustained, and while sterility was not attained in six hours, after 24 hours disinfection was complete. When the concentration of the acriflavine solution added was 0.03 per cent more organisms were present at the end of 24 hours than at the commencement of the experiment. The results with proflavine were not widely different from acriflavine, although the higher concentrations did not effect perfect sterilization in 24 hours. The effect of these dyes appears more pronounced when acting in blood or blood serum than when muscle extract is present.

We have also made some experiments in the progressive change in the bacterial count when various chlorine antiseptics and dyes were added to defibrinated blood. With the exception of the sodium hypochlorite and eusol tests these experiments were made by adding one volume of antiseptic to two volumes of freshly drawn sterile rabbit blood heavily inoculated with *staphylococcus aureus*. The general conditions of the experiments were identical with those preceding.

IV. Chlorine Group of Antiseptics

(Defibrinated Blood Medium; Staphylococcus aureus.)

Antiseptic Used	Concentration		Time of Action	Bacterial Count 1 Drop = $\frac{1}{40}$ cc.
	As Added	In Mixture		
I. Sodium Hypochlorite .	0.5%	0.33%	0 5 min. 15 min. 45 min.	573 400 563 282 2 432
II. do. do.	0.5%	0.3%	0 5 min. 15 min. 45 min.	573 400 1 774 1 485 19 656
III. do. do.	0.5%	0.25%	0 45 min.	232 900 89 600
IV. Eusol	0.27%	0.18%	0 5 min. 15 min. 45 min.	578 600 1 562 2 330 36 600
V. do.	0.27%	0.16%	0 5 min. 15 min. 45 min.	811 000 36 280 48 250 51 580
VI. do.	0.27%	0.13%	0 5 min.	260 800 143 300
VII. Chloramine-T . . .	2%	0.67%	0 5 min. 15 min.	224 500 125 0
VII. do.	1%	0.5%	0 45 min.	235 600 0
VIII. do.	0.75%	0.25%	0 5 min. 15 min. 45 min. 3 hr.	1 178 000 20 160 5 451 4 032 806
IX. Dichloramine-T in Oil	2%	0.67% [1]	0 2 min.	59 900 0

[1] Mixture stirred with platinum wire.

The results of the action of 0.5 per cent sodium hypochlorite and of eusol on staphylococci in a blood medium show that a relatively large addition of these must be made before any marked germicidal effect is produced. Even when two volumes of the antiseptic were added to one of infected blood, complete sterilization was not quite accomplished, although considerably over 99 per cent of the organisms were killed. As already stated, the reason for the apparent low germicidal action of hypochlorites in a blood medium is due to their ready decomposition by the hæmoglobin and other substances in the blood. From a practical standpoint this is not of much importance when methods of intermittent instillation of hypochlorite solution into wounds are practiced but it serves to emphasize the necessity for the frequent renewal of the solution.

Chloramine-T and dichloramine-T give materially better results than the hypochlorites when acting on organisms in a blood medium. A comparison of Experiments I and IX shows that a concentration of 0.25 per cent of chloramine-T in the mixture was as effective as 0.33 per cent sodium hypochlorite, although the active chlorine in the latter was about five times as much as that in the chloramine-T. The reason for this difference is to be sought largely in the slower rate of reaction between chloramine-T and hæmoglobin and other proteins, compared with the hypochlorite solutions.

V. Dyes

(Defibrinated Blood Medium; Staphylococcus aureus.)

Antiseptic Used	Concentration		Time of Action	Bacterial Count 1 Drop = $\frac{1}{40}$ cc.
	As Added	In Mixture		
I. Malachite Green (Grübler)	0.3%	0.1%	0	1 754 000
			5 min.	107
			15 min.	88
			45 min.	66
			1.5 hr.	82
			3 hr.	35
			6 hr.	36
			24 hr.	13
			72 hr.	0
II. do.	0.1%	0.033%	0	1 693 000
			5 min.	1 310 000
			15 min.	819 200
			45 min.	208 000
			1.5 hr.	146 900
			3 hr.	86 010
			6 hr.	89 600
			24 hr.	1 219 000
III. Brilliant Green (Grübler)	0.3%	0.1%	0	1 966 000
			5 min.	0
IV. do. do.	0.1%	0.033%	0	1 630 000
			5 min.	1 261
			15 min.	563
			45 min.	755
			1.5 hr.	717
			3 hr.	857
			6 hr.	2 592
			24 hr.	1 171 000
V. Acriflavine	0.3%	0.1%	0	654 600
			5 min.	358 400
			15 min.	108 800
			45 min.	98 180
			1.5 hr.	1 472
			3 hr.	203
			6 hr.	17
			24 hr.	0

V. DYES — *Continued*

(Defibrinated Blood Medium; Staphylococcus aureus.)

ANTISEPTIC USED	CONCENTRATION		TIME OF ACTION	BACTERIAL COUNT 1 DROP = $\frac{1}{40}$ CC.
	As Added	In Mixture		
VI. Acriflavine	0.1%	0.03%	0	654 700
			5 min.	411 900
			15 min.	225 800
			45 min.	138 100
			1.5 hr.	30 640
			3 hr.	1 152
			6 hr.	32
			24 hr.	0
VII. do.	0.03%	0.01%	0	900 600
			5 min.	819 200
			15 min.	508 900
			45 min.	190 000
			1.5 hr.	83 860
			3 hr.	32 260
			6 hr.	768
			24 hr.	3
VIII. Proflavine	0.3%	0.1%	0	819 200
			5 min.	4 710
			15 min.	1 638
			45 min.	474
			1.5 hr.	304
			3 hr.	113
			6 hr.	12
			24 hr.	0
IX. do.	0.1%	0.033%	0	819 200
			5 min.	201 300
			15 min.	208 000
			45 min.	122 500
			1.5 hr.	53 220
			3 hr.	29 670
			6 hr.	2 995
			24 hr.	2
X. do.	0.03%	0.01%	0	819 200
			5 min.	753 700
			15 min.	852 000
			45 min.	591 400
			1.5 hr.	394 200
			3 hr.	448 000
			6 hr.	48 380
			24 hr.	282

The action of malachite green and brilliant green in blood heavily infected with *staphylococcus aureus* was disappointing. In only one case, namely with brilliant green, did a concentration of 0.1 per cent in the mixture bring about apparent sterility. It is by no means certain that even in this case all the organisms were killed, since it was not possible to avoid carrying over of some of the dye to the agar subcultures, and in the concentration referred to we showed that reproduction of living staphylococci actually was inhibited, although they were not killed. It is significant that with the exception of the two experiments in which brilliant green and malachite green were added in 0.33 per cent solution, an initial fall in the number of bacteria was followed by practically unrestricted growth. These results are in marked contrast to those of Browning and his colleagues referred to in Chapter V. The action of acriflavine and proflavine in blood, while slow, is seen to be distinctly superior to that of the other dyes.

In judging of the suitability of substances for wound treatment, there are of course other factors to be considered besides germicidal efficiency. Prominent among these is the determination of the concentration at which irritation of the skin and other tissues becomes noticeable, also the effect of germicidal substances upon phagocytosis and their influence if any on the rate of dissolution of necrotic tissue. All of these factors may best be investigated *in vivo*. Reference may be made to Colonel Bond's ingenious experiments noted on p. 11 with regard to phagocytic activity as influenced by antiseptics.

Laboratory experiments serve primarily for the sorting out of substances which are likely to be of value for wound antisepsis, and they are particularly important in indicating the mode of use for the selected substance which is most likely to give successful clinical results, but the final decision of the utility of any germicide will necessarily follow from a

study of its effects upon actual wounds. The treatment of comparable infected wounds with various antiseptics, accompanied by daily bacteriological estimations of the degree of infection gives most useful results. This method has been practiced extensively in the present war and gives valuable indications as to the earliest date at which wounds may be safely closed by suture or otherwise. The influence of antiseptics on the rate of cicatrization of wounds may also be followed with the aid of a formula worked out by Du Noüy.[1] The technique of these experimental methods is beyond the scope of this book and reference should be made to original sources.

[1] Journ., Exper. Med., *24*, pp. 451, 461, 1916; *25*, p. 721, 1917.

CHAPTER VIII

CERTAIN SPECIAL APPLICATIONS OF ANTISEPTICS

I. The Disinfection of Carriers

The problem of destroying pathogenic organisms in the nasal and upper air passages by direct disinfection is a difficult one. There are, undoubtedly, many carriers with anatomical abnormalities of the nose, pharyngeal vault and tonsils precluding immediate contact with solutions used either as sprays or gargles. In such cases there is little prospect of any antiseptic proving effective. Those organisms which are exposed to direct contact with the antiseptic solution, may be destroyed, but in the course of time those which have preserved their vitality in protected situations regenerate the original condition and only a temporary, although to that extent beneficial, result is attained. This difficulty is encountered in chronic diphtheria carriers, in whom deep tonsilar crypts are frequently encountered forming inaccessible regions in which the bacilli are afforded protection. The pneumococcus, existing in the pulmonary air passages, is, in those parts, wholly beyond reach. In considering the applicability of treatment to individual cases, these inevitable limitations must be taken into account.

In spite of these limitations, chiefly imposed by anatomical conditions, valuable results have been obtained, notably by Gordon and Flack, with the use of antiseptics in the treatment of meningococcus carriers among soldiers. By a suitable choice of antiseptic, properly applied, there is no doubt that much can be accomplished in limiting the risks

connected with carriers of pathogenic organisms, both to themselves and those coming in contact with them.

The methods that have been employed with most success are the following:

(1) Chamber disinfection using a steam spray and a solution of chloramine-T or zinc sulphate (Gordon and Flack). Hypochlorites have also been used for chamber disinfection, but they have not been found as satisfactory.[1]

(2) Local application of various antiseptics including iodine, with either menthol, guaiacol or glycerine, argyrol and hydrogen peroxide, zinc salts, acriflavine, chloramine-T and especially dichloramine-T in oil solution. Ferric chloride and potassium permanganate solutions have given only moderate results while formalin appears to be distinctly harmful.

Gordon and Flack found that many of the antiseptic solutions employed as gargles and in hand sprays at the time when they commenced their experiments gave unsatisfactory results when used on heavily infected chronic carriers. This appeared to be due as much to lack of perfect contact as to germicidal inefficiency. They therefore employed an inhalation chamber with a steam pressure spray or atomizer capable of converting the antiseptic solution into a fine mist. The chamber was about 750 to 1000 cubic feet capacity, and the apparatus was capable of spraying a liter of antiseptic solution in the course of twenty minutes. A working diagram of two forms of these sprays will be found in Gordon and Flack's original paper,[2] in which there will also be found details of the successful treatment of numbers of chronic meningococcus carriers. The prepared antiseptic solution used for spraying by Gordon and Flack was either chloramine-T, 0.5 per cent, or zinc sulphate, 1.2 per cent. The chloramine-T gave the better results, especially with persistent carriers, but is somewhat less well

[1] Kuster, Deutsch. Med. Woch, *41*, p. 1116, 1915.
[2] Brit. Med. Journ., p. 673, Nov. 18, 1916.

tolerated than the zinc salt. The inhalation was carried out once daily and on each occasion the carriers were in the inhaling room from fifteen to twenty minutes during which time they vigorously inhaled the spray-laden atmosphere through the nostrils.

Using a different apparatus with a high pressure air spray, the authors [1] have examined the antiseptic action of chloramine-T solution on the mixed bacterial flora of the normal nasopharynx. The results are recorded in the following table in which, for comparison, the effect of spraying with salt solution is included. The figures, which are the average of six similar experiments, indicate the total number of organisms obtained from plating a swab taken under approximately constant conditions and in which any antiseptic adhering to the swab was immediately destroyed with sterile sodium thiosulphate solution.

AQUEOUS CHLORAMINE-T AND NEUTRAL SALT SOLUTION

	NUMBER OF COLONIES DERIVED FROM SAMPLE					
	First Period			Second Period		
TIME OF TREATMENT	5 min.	10 min.	15 min.	5 min.	10 min.	15 min.
0.5% chloramine-T (control, 43.240)	1,331	707	526	439	153	17
Normal salt solution (control, 56.738)	12,352	121,620	13,587	2,137	10,055	34,339

From these and other results, it appeared that exposure to the chloramine-T spray for less than fifteen minutes was not very effective and that frequent repetition of the treatment was desirable. Repetition several times daily of the

[1] Brit. Med. Journ., June 23, 1917.

chloramine-T spray for 15 or 20 minute periods is rather a severe procedure, so that as an alternative we have employed an oily solution of the related dichloramine-T (see p. 33), which has the great advantage that its action is much more prolonged, owing to the slow diffusion of the antiseptic into the aqueous nasal secretions. The oil solution is sprayed into the nose and throat from an ordinary oil spray, preferably one made entirely of glass, thus avoiding the necessity of an inhalation chamber.

Treatment Employed	Applications of Oil between Tests	Time of Test, from Beginning of Treatment	Number of Colonies on Agar Plate
I. 0.5% aq. chloramine-T followed by 2% di-chloramine-T in oil.	0	0	42,240
	1	30 min.	9
	0	60 min.	0
II. 1.5% dichloramine-T in oil.	0	0	16,104
	1	1 hour	175
	1	3.5 hours	1
	0	6 hours	9
III. 0.5% aq. chloramine-T followed by 1.3% dichloramine-T in oil.	0	0	6,129
	1	1 hour	8,960
	4	4 hours	87
	0	20 hours	1,980
	1	22 hours	59
	1	24 hours	3
	1	26 hours	1
	1	27.5 hours	20

Experience in the treatment of meningococcus carriers with 2 per cent dichloramine-T dissolved in chlorinated eucalyptol and paraffin (1 : 4) has as yet been limited, but many cases treated have been promptly freed from the meningococcus and the method appears likely to be used extensively. The above table shows the effect of spraying the oil solution of dichloramine-T on the normal nose. In such experiments it must be recalled that many of the bac-

teria are much more resistant to antiseptics than the meningococcus and that spore forms were probably present.

In the above experiments, the strongest solution was 2 per cent and the result was very striking. It should be understood, however, that the concentration of the antiseptic in the oil bears no simple relation to the strength actually applied to the organisms in the aqueous secretions. For example, a sample of the oil containing 2 per cent dichloramine-T, when shaken with an equal volume of clear nasal secretion, showed that the filtered aqueous portion had a concentration of active chlorine corresponding to about 0.1 per cent dichloramine-T. The chief advantage of using a concentrated oil solution lies in prolonging the period through which it can serve as a store of active antiseptic. In the normal nose, a 2 per cent solution is not exhausted until about two hours have elapsed; but when foul discharges are present, this period would be curtailed.

The experience hitherto gained in the treatment of carriers with dichloramine-T indicates that the following procedure may be advantageously followed:

First cleanse the nose with normal salt solution, with or without the addition of 0.25 per cent chloramine-T, by spraying, or irrigation. The same chloramine-T solution should be used as a gargle. After this preliminary treatment and when the augmentation of nasal secretion has subsided, apply the oil solution of dichloramine-T [1] (2 per cent) with an oil "atomizer," endeavoring to reach all the parts with an abundant supply of oil. It is not certain that the oil so introduced is ever active for more than two hours, so that for intensive treatment it should be renewed at the end of

[1] This solution is best prepared by dissolving 0.2 gram of dichloramine-T in 10 cc. chlorcosane rendered more fluid, if need be, with 1 cc. carbon tetrachloride. The solution should be discarded as soon as signs of a considerable crystalline sediment appear. Ordinarily it is stable for several weeks. It should be carefully protected as far as possible from direct exposure to sunlight, as this hastens its decomposition.

that time. In any case, it appears important to repeat spraying with the oil so that four treatments a day are given at about equal intervals. The first few applications of oil sometimes occasion sneezing, but the nose appears to acquire a tolerance for the treatment, and subsequent applications occasion no inconvenience. In cases of acute coryza, dichloramine-T applied as described is too irritating to the inflamed membrane and its use is not advised.

In the way of prophylaxis, spraying of the upper air passages with a 0.25 per cent solution of chloramine-T in saline, or an oil solution of dichloramine-T appears to be followed by good results in reducing the incidence of coryza, measles and mumps, although it is not possible to cite convincing statistical evidence. Colonel Parke's [1] report on army transports covering long voyages in which an inhalatorium of the Gordon and Flack type was used, lends support to the view that such treatment is useful. The simplicity of these methods and the apparent value of the results indicate that more extended trials are desirable.

Among the other antiseptic solutions employed in the treatment of meningococcus carriers, reference may be made to an iodine and menthol preparation frequently employed in England, while Vincent in the French army advocated the following mixture as an inhalation: iodine 12 grams, potassium iodide 6 grams, guaiacol 2 grams, thymol 0.35 gram, alcohol 200 grams, used five or six times a day. For disinfection of the pharynx, swabbing with a 3 to 5 per cent solution of glycerine and iodine is advocated. This would appear a decidedly drastic treatment. Sophian has recommended hydrogen peroxide 1 per cent and argyrol 9 per cent, used as a spray, as of value for freeing carriers of meningococci, although the mixture does not seem to have been used extensively in military centres.

The antiseptic treatment of carriers has been much more

[1] Brit. Med. Journ., Feb. 24, 1917.

successful in meningococcus cases than with most other infections. The reasons for this are doubtless due in large measure to the fact that the meningococcus is very readily killed by antiseptics and is usually found in situations accessible to treatment. The treatment of diphtheria carriers has been less successful, though encouraging, while pneumococcus carriers give disappointing results. The reasons for this are obvious when the pathological conditions are considered.

II. The Disinfection of Water

The chemical disinfection on a large scale of contaminated or suspected water supplies for civilian military or naval use is now almost exclusively effected with substances belonging to the chlorine group.[1] When working with considerable volumes of water, the choice of reagent is practically limited to free chlorine supplied from cylinders of the liquefied gas, sodium hypochlorite solutions of known concentration, or bleaching powder (chloride of lime). The action of these substances is essentially similar, and their usefulness depends upon the fact that their addition in such quantity that about 0.5 to 1 part of active chlorine per million parts of water persists for a short period, is followed by an almost complete disappearance of organisms of the coli group. Spore forms which are of little hygienic importance are not much affected. Preliminary filtration of turbid waters is desirable, and occasionally in the case of extreme turbid waters, clarifying with some precipitant, such as alum followed by alkali, is resorted to.

The choice of reagent will depend largely upon the actual conditions of its employment. When large volumes of water are to be treated and liquefied chlorine is available,

[1] An old chemical method of purifying water depended on the use of potassium permanganate. Except against the cholera spirillum, it is not very effective, and the cost of the material, and the disagreeable appearance and taste of the treated water, are serious drawbacks.

this may be the most convenient method, and especially if a gas gauge such as the Wallace-Tiernan is employed by means of which the desired chlorine concentration in a known quantity of water may be easily regulated. Sodium hypochlorite solution is preferable to bleaching powder in that it is more readily distributed through the water, but its varying composition and instability make it less satisfactory for field use. For the sterilization of moderate volumes of water such as those contained in army water carts, *e.g.* 110 gallons, bleaching powder has given most satisfactory results.

The successful use of chlorine compounds for the disinfection of water depends largely upon adequate mixing with the water; the use of an effective concentration of disinfectant such as that already indicated; and lastly the avoidance of excess. The taste and smell of unnecessarily highly chlorinated water is objectionable to most people. Not infrequently a reducing substance such as sodium thiosulphate is added to remove the excess of chlorine, but with careful chlorination this should not be necessary. The exact amount of disinfectant to be added to water can only be fixed with regard to individual supplies of constant composition. The reason for this is that different waters contain such varying amounts of organic matter and sometimes ferrous salts or sulphides as well, and the chlorine used up in oxidizing these substances will not effect sterilization. A slight excess only of the disinfectant must therefore be employed, and the proper amount is most readily determined by adding a little more (*e.g.* 0.5 to 1 per million) than the minimum quantity necessary for obtaining a positive reaction for active chlorine in the water. The test for active chlorine is most easily carried out by adding a few drops each of 10 per cent potassium iodide solution and starch paste to about 200 cc. of water and noting whether a blue color develops. Since starch paste is apt to become mouldy, it is

often convenient to use zinc iodide (7.5 per cent) in place of the potassium salt and to add this directly to the starch paste together with two per cent of alcohol. This mixture keeps well. Various simple devices have been used for carrying out this test in the field so that accurate dosage may be rapidly determined. It should be remembered that the sensitiveness of the starch-iodide reaction for active chlorine rapidly diminishes with rise in temperature of the water. The speed with which the reaction for active chlorine disappears from a treated water depends upon many factors, such as the degree of surface exposure, carbon dioxide concentration, the presence or absence of much ammonia in the water, and the nature and amount of the organic matter. Under most circumstances, an initial concentration of 0.5 part per million of active chlorine will vanish in a very few hours.

It has been observed that if an addition of ammonia is made to the hypochlorite to be used for water sterilization, the resulting product monochloramine, NH_2Cl, is still more active as a disinfectant than the original hypochlorite,[1] and its action is more prolonged. Use of this fact has been made in the sterilization of the water supplies of several large communities with excellent results. A concentration of 0.6 part per million of active chlorine, used as monochloramine, appears to give a considerable margin of safety.

Further details concerning the large scale disinfection of water should be sought in books dealing primarily with questions of hygiene.

THE DISINFECTION OF SMALL QUANTITIES OF WATER. — Chlorine gas, bleaching powder or sodium hypochlorite, which are so effective for the disinfection of relatively large volumes of water, are not suitable for the individual sterilization of small quantities such as the contents of an army water bottle.

[1] Rideal, Journ. Roy. Sanitary Inst., *31*, p. 33, 1910.

The problem of sterilizing small individual quantities of water such as are needed by cavalry or rapidly moving troops is a difficult one, for the use of the chlorine antiseptics just mentioned is generally impracticable owing to the instability of small tablets containing the required minute quantity of active disinfectant. In their place acid sulphates of the alkali metals have been used to a considerable extent, and these will be considered later; but the superior potency of the chlorine antiseptics induced us to try and find suitably stable compounds, and one of these which will now be described has met with some success as the result of practical trials.

A number of compounds which at first sight seemed likely to be useful had to be discarded either because they were ineffective in hard waters or because of difficulties concerning solubility or stability. The most suitable substance that we have as yet found is p-sulphondichloraminobenzoic acid, — $Cl_2N{\cdot}O_2S{\cdot}C_6H_4{\cdot}COOH$. It is easily prepared from cheap, readily available materials, and appears to be effective and reasonably stable. The presence of the COOH group confers a slight but definite degree of solubility in water, which is increased by dispensing it with alkaline salts such as sodium carbonate or borax. Formulæ for the tablets are appended together with details of the preparation of the substance and an estimate of its cost. Since the systematic name of the disinfectant is inconveniently long for ordinary use, we have adopted the name "halazone." The abbreviated name gives some indication of the character of the compound.

In the following table are given some of the bacteriological results. The technique employed was that in general use and requires no special description. Five or ten standard drops of the treated water were generally used for plating on agar for counting the surviving organisms. Suitable controls were invariably carried out.

EXPERIMENTS WITH p-SULPHONDICHLORAMINOBENZOIC ACID IN TABLET FORM.

No.	WATER TREATED	CONCENTRATION OF DISINFECTANT	TIME OF ACTION IN MINUTES	SURVIVING ORGANISMS PER C.C.	TABLET DISSOLVED IN MINUTES
1	Tap water B. coli.	0	—	112,525	
		1 : 400,000	15	0	10
2	Tap water B. coli.	0	—	178,528	
		1 : 500,000	15	987	10
		1 : 500,000	30	0	10
3	Tap water 5% sewage B. coli.	0	—	1,119,000	
		1 : 175,000	15	6,000	9
			30	0	9
			60	0	9
4	Tap water 5% sewage B. coli.	0	—	1,158,500	
		1 : 330,000	15	120,064	7
		1 : 330,000	30	9,146	7
		1 : 330,000	60	0	7
5	Deep yellow polluted river water B. coli.	0	—	33,152	
		1 : 250,000	20	10,940	13
		1 : 250,000	40	852	
		1 : 250,000	60	0	
6	Tap water B. typhosus.	0	—	1,155,400	
		1 : 225,000	20	0	17
7	Hard water B. typhosus.	0	—	85,400	—
		1 : 333,333	20	0	14
8	Tap water 5% sewage B. typhosus.	0	—	66,017	
		1 : 333,333	20	242	14
		1 : 333,333	40	0	
9	Tap water B. paratyphosus A.	0	—	112,000	
		1 : 225,000	20	0	17
10	Tap water 5% sewage B. paratyphosus A.	0	—	29,400	—
		1 : 333,333	20	15	12
		1 : 333,333	40	0	
11	Tap water B. paratyphosus B.	0	—	322,500	
		1 : 225,000	20	10	17
		1 : 225,000	40	0	

EXPERIMENTS WITH p-SULPHONDICHLORAMINOBENZOIC ACID IN TABLET FORM. — *Continued.*

No.	WATER TREATED	CONCENTRATION OF DISINFECTANT	TIME OF ACTION IN MINUTES	SURVIVING ORGANISMS PER C.C.	TABLET DISSOLVED IN MINUTES
12	Tap water 5% sewage B. paratyphosus B.	0	—	130,776	
		1 : 333,333	20	290	12
		1 : 333,333	40	0	
13	Tap water V. choleræ.	0	—	13,706	
		1 : 450,000	20	0	13½
14	Tap water 5% Sewage V. Choleræ.	0	—	11,170	13
		1 : 333,333	20	0	
15	Tap water B. dysenteriæ (Flexner).	0	—	66,998	
		1 : 450,000	15	25	13
		1 : 450,000	30	0	
16	Tap water 5% sewage B. dysenteriæ (Flexner).	0	—	164,864	
		1 : 333,333	20	38	13
		1 : 333,333	40	0	
17	Tap water B. dysenteriæ (Shiga).	0	—	31,200	
		1 : 333,333	20	10,934	11
		1 : 333,333	40	0	
18	Tap water 5% sewage B. dysenteriæ (Shiga).	0	—	2,108	
		1 : 333,333	20	0	13

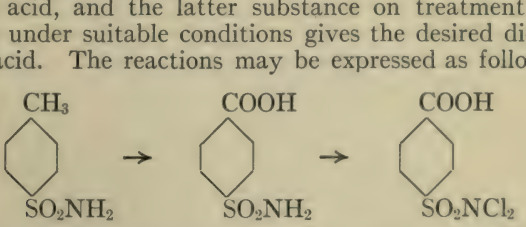
From the results in the table it appears that a concentration of 1 : 300,000 is sufficient to sterilize an ordinarily heavily contaminated water in about thirty minutes. Such a concentration could be relied upon to remove coli, typhoid, or cholera organisms. Special experiments showed that the substance in tablet form was efficacious when acting on water contained in aluminium bottles, although a very trifling action on the metal may be observed if tablets are allowed to remain undisturbed in long contact with the metal. We

believe such action to be of no practical moment. The concentration of disinfectant given above is just perceptible to the taste, especially in warm water containing little organic matter, but the water is perfectly palatable. Tea brewed with or without the addition of halazone to the water cannot be distinguished. One point of advantage possessed by the present disinfectant over most hypochlorite preparations is the fact that the active chlorine is less rapidly used up, so that the process of disinfection continues for a longer period.[1]

Even in large doses the substance is not toxic (250 milligrams per kilo) and is excreted by rabbits in the urine as p-sulphonamidobenzoic acid.

PREPARATION AND PROPERTIES OF HALAZONE. — The starting point in the preparation of the substance is p-toluenesulphonamide, a product which is readily obtained by the action of ammonia on p-toluenesulphonic chloride. The latter substance is a cheap waste product in the manufacture of saccharine, and is available in relatively large quantities. It is also used for the manufacture of chloramine-T, and dichloramine-T.

Toluenesulphonamide is oxidized to p-sulphonamidobenzoic acid, and the latter substance on treatment with chlorine under suitable conditions gives the desired dichloramino acid. The reactions may be expressed as follows:



The experimental details are as follows: Add 250 grams commercial sodium dichromate to a mixture of 200 cc.

[1] It may be noted that "halazone" is unsuited for use as a preservative for milk or other foodstuffs, or as a general antiseptic.

sulphuric acid and 600 cc. water contained in a 2 liter round flask. Then add 100 grams crude toluene-p-sulphonamide and heat on a sand bath with reflux condenser for one hour, using a small flame at first as the reaction is vigorous. On cooling wash the separated crystals well with cold water, and then dissolve them in hot dilute sodium hydroxide in slight excess. Filter hot, and add excess of hydrochloric acid and when cold filter off the precipitated sulphonamidobenzoic acid, wash well with water and dry. The yield is about 80 per cent of theory.

Twenty grams of p-sulphonamidobenzoic acid are dissolved in 200 cc. approximately normal sodium hydroxide (2 mols.) warming if necessary. About 200 grams of crushed ice is then added, and the mixture saturated with a rapid current of chlorine. The reaction is most conveniently carried out in a fairly wide-mouthed flask, which may be shaken while the gas is being introduced. If the temperature should rise more ice can be added. A white, rather chalky precipitate of the dichloramino acid is at once precipitated. The acid is filtered off, using suction, well washed with cold water, and dried in vacuo on a porous plate. The dry substance is practically pure, and may be powdered and preserved apparently indefinitely. Prepared by this method, the yield of the dichloramino acid is practically the theoretical amount.

The substance is sparingly soluble in water and in chloroform and insoluble in petroleum. It readily dissolves in glacial acetic acid, crystallizing in stout prisms, which melt at 213° C. The substance explodes feebly when rapidly heated on platinum foil, but, compared with most members of the group, is remarkably stable.

The purity of the compound may be checked by titration as follows: 0.1 gram is weighed out, dissolved in 50 per cent acetic acid and potassium iodide added. The liberated iodine is titrated with decinormal sodium thiosulphate, of which 14.8 to 14.9 cc. will be required (see p. 40).

The dichloramino acid dissolves, apparently without change, in excess of cold sodium hydrate solution, and may be reprecipitated on addition of acids. With smaller quantities of sodium hydroxide or with feebly alkaline salts, such as phosphates or borates, hydrolysis occurs, with liberation of disagreeably smelling compounds of nitrogen and chlorine.

Dry sodium carbonate or dry borax appear the most satisfactory alkaline salts for making tablets containing halazone. A convenient formula for tablets weighing 100 to 105 mg. is to use sulphondichloraminobenzoic acid 4 per cent, sodium carbonate 4 per cent (or dried borax 8 per cent) sodium chloride (pure) 92 per cent. The acid should be ground up with the dry salt and the sodium carbonate or borax added subsequently. The mixture, which must be kept perfectly dry, may be passed through a 40 mesh sieve. No lubricant or other addition is necessary and should be avoided. The strength of the tablets should be tested by dissolving in 50 per cent acetic acid and potassium iodide solution, and titrating with decinormal sodium thiosulphate as already described (p. 40) (1 cc. of N/10 thiosulphate = 0.00675 gram of the dichloramino acid). They must not be allowed to dissolve in water and subsequently titrated, as then decomposition occurs. The tablets should be stored in small amber glass bottles, to protect them from the action of light. Tablets so prepared of the weight mentioned contain about 4 mg. of the disinfectant, and are suitable for the sterilization of a liter or quart of reasonably heavily contaminated water. In the case of extreme contamination a second tablet may be necessary.[1]

The practical success of the disinfectant depends very largely on the stability of the tablet. It appears, as judged by several months' observation, that the tablets are quite stable enough for practical use. They are certainly more

[1] Halazone tablets can be obtained from Boots, Island Street, Nottingham, England, or Abbots of Chicago and doubtless other firms.

permanent than other similar compounds with which we are acquainted, and when kept in amber bottles under ordinary conditions, and at temperatures not exceeding 30°C., less than 5 per cent decomposition was observed in 150 days.

It is rather difficult to give precise estimates of the cost of the finished product, but it is safe to say that the tablets could be sold at such a price that 100 gallons of water could be sterilized at a cost of one penny.

Sodium Bisulphate and Other Acid Substances. — The injurious effect of acids upon the cholera vibrio and typhoid bacillus was pointed out long ago by Koch and Kitasato, Stutzen, and others. From Kitasato's experiments it appeared that from about 0.2 to 0.5 per cent of most acids was necessary to free water from typhoid bacilli in a few hours, but that a notably lower concentration of sulphuric acid was effective. The following table has been compiled by Colonel Horrocks, illustrating the effect of various acid substances upon typhoid bacilli.

Acids	Per Cents of Reagent		
	Growth	Growth Restrained	No Growth
Sulphuric Acid	0.049	0.065	0.08
Hydrochloric Acid	0.1	0.158	0.2
Nitric Acid	0.1	0.157	0.2
Sulphurous Acid	0.09	0.2	0.28
Phosphoric Acid	0.15	0.224	0.3
Acetic Acid	0.2	0.255	0.3
Formic Acid	0.22	0.278	0.34
Oxalic Acid	0.23	0.285	0.366
Lactic Acid	0.27	0.36	0.4
Tartaric Acid	0.338	0.384	0.476
Citric Acid	0.338	0.384	0.476
Malic Acid	0.338	0.384	0.476
Tannic Acid	1.3		1 66
Boric Acid	1.5	2.0	2.7

In these experiments broth and gelatine were carefully neutralized before adding the substances, then inoculated with *B. typhosus* and kept at room temperature, subcultures being taken at 4-5 and 10-15 hours. Considerably greater disinfecting action was obtained with waters containing a minimum of organic matter.

The possibility of using an acid mixture for the sterilization of small quantities of water was put into practical form by the suggestion that solid tablets of acid sodium sulphate might be employed. From the experiments of Rideal [1] and Parkes it appears that one gram of sodium bisulphate per pint of water is adequate for the sterilization of waters moderately heavily contaminated with typhoid bacilli in fifteen minutes, while very heavily infected waters may require forty-five minutes. Under similar conditions *B. enteritidis* is destroyed about as readily as *B. typhosus* while the *V. cholerœ* is more sensitive than either.

Sodium bisulphate tablets were used by the New Zealand contingent in the South African War and subsequently to some extent by cavalry and mounted police and other mobile forces. They are frequently compounded with lemon oil and saccharin so that the resulting solution has some resemblance to lemonade. The taste of this acid mixture is actively disliked by some individuals but well tolerated by others. The acid sulphates have the disadvantage of attacking metallic water bottles to a marked extent and while undoubtedly useful, do not possess the potency or security afforded by a disinfectant of the chlorine group. The tablets apart from mechanical disintegration have the advantage of being indefinitely stable.

[1] Rideal, Disinfection and the Preservation of Food, John Wiley & Sons, 1903, p. 376.

III. The Disinfection of Hospital Ships, etc., with Sodium Hypochlorite Prepared by Electrolysis of Sea Water or Brine

In the following section an account is given of a relatively simple and cheap apparatus for the preparation of considerable quantities of sodium hypochlorite by the electrolysis of sea water. The results obtained by the use of hypochlorite disinfectant in reducing secondary infections on large hospital ships carrying many infectious cases, have been sufficiently striking to justify, perhaps, the inclusion of an abridged report on the use of this apparatus.

Opportunities for the employment of the apparatus are by no means restricted to hospital ships for the only requisites are salt water or brine and a supply of direct current electrical energy.

The preparation of sodium hypochlorite by the electrolysis of salt solutions is an old process, and since Watt's initial discovery in 1859 innumerable modifications in its mode of production have been introduced, chiefly for industrial purposes. The deodorizing and germicidal properties of the hypochlorites have been known for more than a century, and it is impossible to refer to many of the applications for hygienic purposes which these substances have found.

Reference may be made, however, to the well-known Hermite process for the sterilization of sewage by electrolytic hypochlorite, introduced about 1893; to the wide employment of hypochlorites for the purification of suspected potable waters, and to the many uses as disinfectant which hypochlorites have found in sanitary work. Electrolytic hypochlorite for general disinfection purposes has been successfully employed by a great many sanitary authorities.

The apparatus to be described was originally employed on the Hospital Ship "Aquitania" and since then has been

adopted on a number of other ships and coast stations.[1] It was first of all necessary to devise an apparatus suitable for use on shipboard, then to determine favorable conditions for carrying out the electrolysis and to decide on suitable methods for applying the product. Nothing essentially new is embodied in the design or use of the apparatus but many existing electrolyzers are either unsuitable or subject to patent restrictions which limit their usefulness.

DESCRIPTION OF APPARATUS. — The necessary apparatus comprises an electrolytic cell, a reversing switch capable of carrying a hundred amperes and some ordinary insulated electric cable. A scale drawing of the electrolytic cell is given on the following pages.

The electrolyzer consists of a rectangular box made of teak or cedar securely bolted together. It may be coated internally with marine glue to protect the wood and to reduce the risk of leaking. The interior of the box is divided into twenty or preferably twenty-five cells by means of carbon plates placed parallel to one another. The plates situated at both ends of the box project above the level of the other plates to receive the terminals for the introduction of the current. The terminal electrodes for convenience are made in four pieces placed side by side, while the intermediate carbons are made in three parts superimposed vertically one upon the other. A copper plate attached to the four binding screws at each end insures an even distribution of current.

The carbon plates are separated from each other by strips of vulcanite or wood, or glass tubes, and they are kept in position by means of a "making up block" and wedges placed at one end of the tank. A grooved channel is cut along one side of the floor of the wooden tank underneath the carbon plates in order to facilitate emptying by means of a wooden or vulcanite plug tap inserted in a hole con-

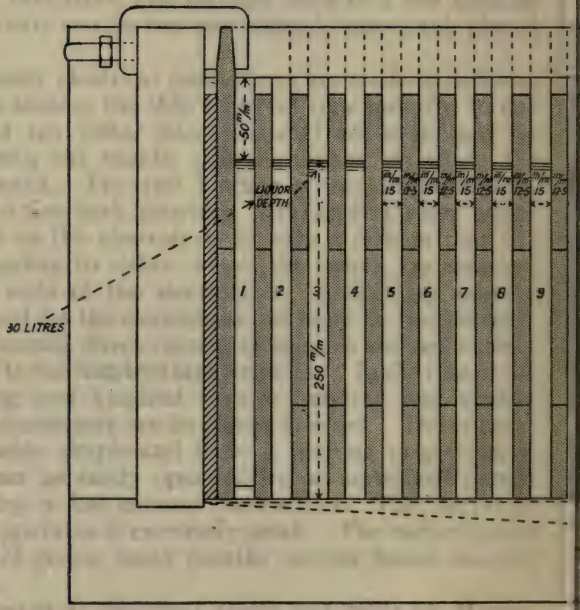
[1] Dakin and Carlisle, Jour. Roy. Army Medical Corps, Feb., 1916.

nected with the floor of the tank. A word must be added as to the necessity of employing suitable carbon plates. These should be of Acheson graphite prepared by treating amorphous carbon in the electric furnace. Ordinary carbon plates rapidly deteriorate and cannot be used satisfactorily. The tank should be mounted on a rubber mat or on glass or porcelain insulators, and securely fixed to a low table so that its contents can be run conveniently into a tub placed beneath it.

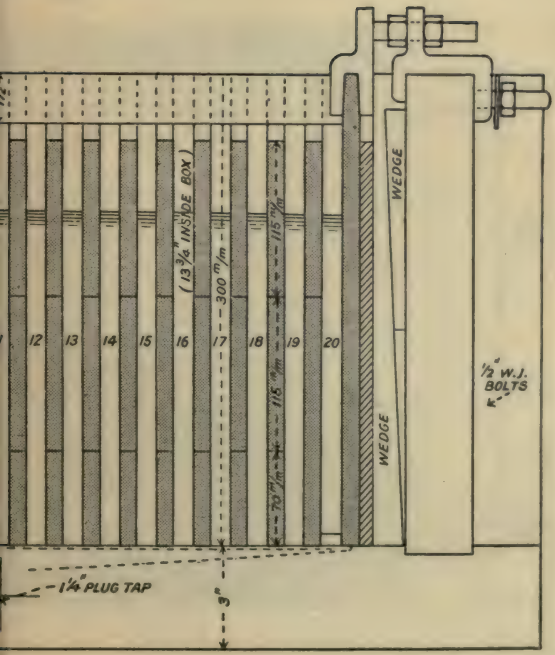
The necessary electrical connections are made as follows: — two wires leading the ship's current (one hundred to one hundred and ten volts, direct current) are connected respectively with the middle pair of binding screws in the reversing switch. The two binding screws on both sides of the switch are each provided with leading wires which are attached to the electrolyzer in such a fashion that the two wires leading to either side of the switch are attached to opposite ends of the electrolyzer. Alternating current cannot be used for the electrolysis but must be transformed. Most ships furnish direct current of suitable voltage, namely one hundred to one hundred and ten volts. In the case of the voltage being two hundred to two hundred and twenty volts, two electrolyzers can be placed in series. The apparatus is extremely simple and there is nothing to get out of order. It can be easily operated by an untrained person after receiving a few minutes' instruction. The wear and tear of the apparatus is extremely small. The carbon plates are capable of giving many months' service before renewal is necessary.

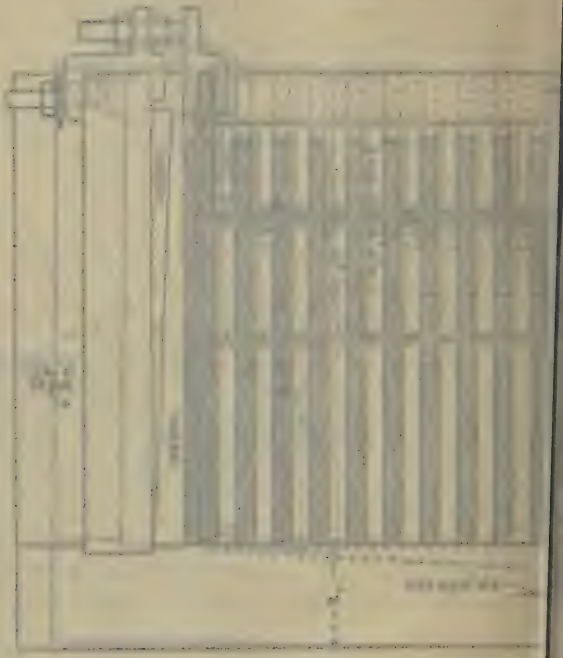
The apparatus as described above was made by Messrs. Mather and Platt, Park Works, Manchester. We are indebted to Dr. Edward Hopkinson, a director of the company, for much valuable assistance in constructing a practical and efficient apparatus. The makers' number for the first electrolyzer of this type is G.M. 718/15, and may be usefully

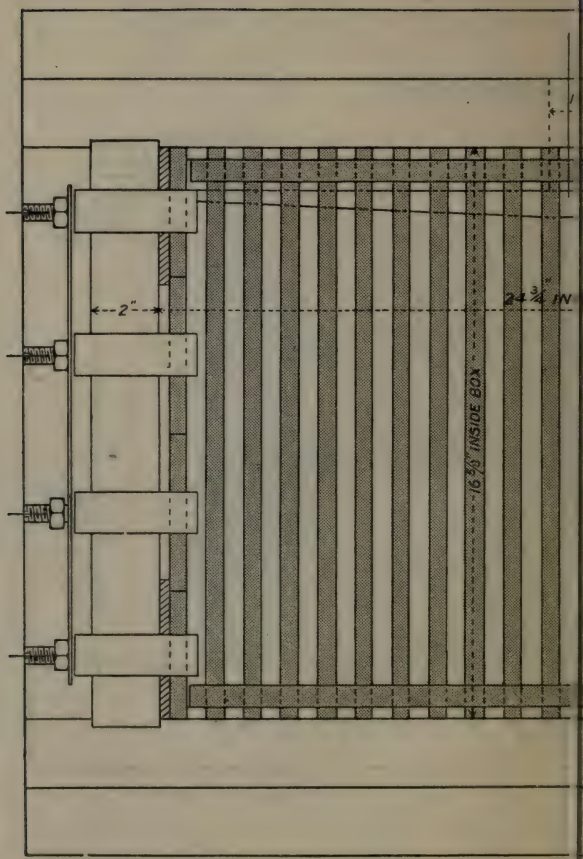
20-CELL SPECIAL

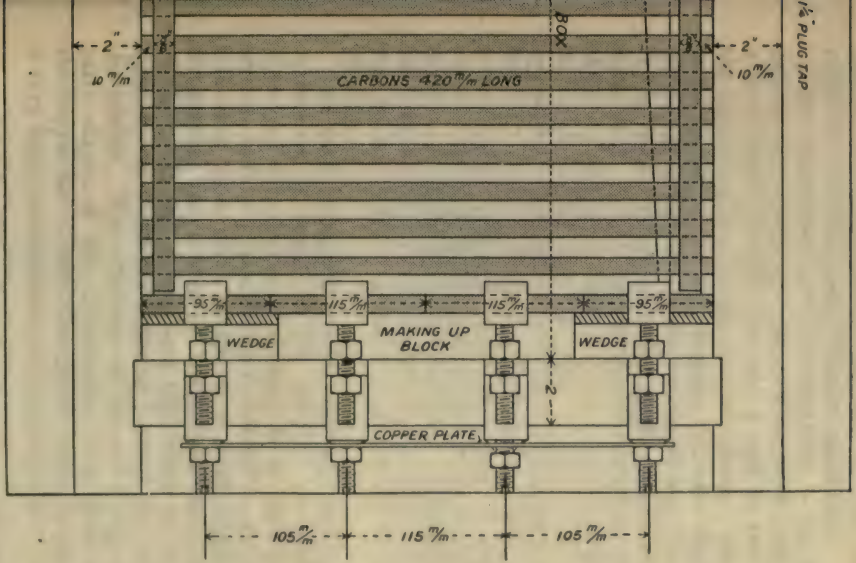


LECTROLYSER.






2"
24 3/4" IN
16 5/8" INSIDE BOX


2"
10 m/m
CARBONS 420 m/m LONG
BOX
1/4" PLUG TAP
95 m/m
115 m/m
115 m/m
95 m/m
WEDGE
MAKING UP BLOCK
WEDGE
2"
COPPER PLATE
105 m/m
115 m/m
105 m/m

quoted for identification purposes in any correspondence relative to the purchase of similar apparatus.

THE OPERATION OF ELECTROLYSIS. — In order to carry out an electrolysis the tank is filled with cold salt water to a level approximately one and a half to two inches below the upper surface of the carbon plates. The object is to avoid excessive current leakage between the cells such as would occur if the sea water reached a level above the plates. If the ship is rolling, less sea water should be used at each operation. A tank of the dimensions given will hold about thirty to thirty-five liters, or six to seven gallons. The salt water should be cold in order to limit the conversion of hypochlorite into useless chlorate consequent upon excessive heating. Special experiments made with sea water at temperatures varying from 5° C. to 25° C. showed that the cell can electrolyze sea water efficiently at any temperature likely to be encountered in practice. During the electrolysis the temperature of the solution in the cells rises about 1.5° C. per minute when a current of about 50 to 70 amperes is passing.

The tank being filled with cold salt water the current is turned on by means of the switch.[1] A brisk evolution of hydrogen with traces of chlorine is at once noticeable, and neutral sodium hypochlorite is formed in the solution. The following table gives details of a single typical experiment illustrating the course of electrolysis after varying lengths of time. It will be noticed that after a few minutes the quantity of available chlorine formed per minute steadily diminishes. The efficiency of the cell being greatest in the early stages of the electrolysis, it is most economical to electrolyze for a short time rather than to aim at attaining a very high concentration of hypochlorite. Using ordinary

[1] Untrained attendants must be warned against turning the current on before filling the tank and against adding salt water to the tank while electrolysis is in progress.

salt water, it is inadvisable to continue the electrolysis for more than ten minutes. With a current of 60 to 75 amperes at 110 volts, ten minutes' electrolysis will give a solution usually containing a little more than four parts per thousand of sodium hypochlorite or of available chlorine. For most purposes it is distinctly advantageous to limit the electrolysis to five minutes. Under these conditions the efficiency of the cell is high and the rise of the temperature of the solution is not great — about 7° to 8°. The solution at the end of five minutes will contain from 2.0 to 2.7 parts per thousand of sodium hypochlorite or available chlorine.

Time of Electrolysis Minutes	Available Chlorine			Additional Data
	Grams per Liter	Yield per Minute Stage Grams per Liter	Total Available Chlorine Produced Grams	
1	0.41	0.41		
2	0.92	0.51		
3	1.49	0.57		35 liters sea water.
4	2.08	0.59		Temperature at start, 15° C.
5	2.66	0.58	141.4	Temperature at finish, 28° C.
6	3.09	0.43		Temperature rise, 13° C.
7	3.37	0.28		Mean voltage 110 ± 1
8	3.64	0.27		Mean amperage, 72.
9	3.90	0.26		
10	4.04	0.14		

The direction of the current should be reversed from time to time by turning the handle of the reversing switch in the opposite direction to that previously used. A daily reversal is probably sufficient, but it may be done more often with advantage. Current reversal keeps the carbon plates free from deposits of magnesium hydroxide, which otherwise would lower the efficiency of the cell.

When less current than sixty amperes is taken by the cell, the yield of hypochlorite is naturally lower.

With approximately constant current the yield of hypochlorite is remarkably uniform so far as practical purposes are concerned. Where higher concentrations of hypochlorite are required, they may be obtained by electrolyzing cooled brine containing ten or fifteen per cent of salt.

Apart from a few particles of suspended carbon from the electrode, the electrolyzed solution will be found clear, bright, and free from sediment. It should be run off into wooden tubs or other receptacles, such as slate or lead-lined vats.

It is desirable to make a few tests of the output of hypochlorite on installing a new apparatus on board ship, especially when the current available differs greatly from that indicated. This, however, is a very easy matter. (See page 41.)

Cost of Apparatus and of Electrolytic Hypochlorite. — The cost of disinfection with electrolytic chlorine may be resolved into two items: (1) the initial cost of the cell, and (2) the cost of the current used for electrolyzing the sea water.

From an examination of the results recorded opposite, it is calculated that when electrolyzing 35 liters of sea water at a time for five-minute periods, using 110 volts and 75 amperes, the power required to produce a kilogram of chlorine is approximately eight kilowatt hours. The cost of the electrical energy naturally varies enormously according to the conditions of its production — the estimates varying from 0.25 pence to 1.5 pence per unit. Taking 0.75 pence per unit as a fair average, the cost of producing 1000 liters of hypochlorite solution at 1 : 1000 chlorine concentration works out at sixpence. This is equivalent to threepence per 100 gallons, a figure which for practical purposes may be regarded as almost negligible.

The original cost of the cell installed on shipboard is about £ 50, and its depreciation is undoubtedly small. The carbon plates will last for over a year with constant use and hence much longer when intermittently worked, as on board ship. Moreover, they can be readily replaced at moderate cost. The tank itself, being of teak or cedar wood, can be easily repaired in case of need by the ship's carpenters.

Judging by certain estimates supplied to us, it is calculated that on a ship the size of H.M.H.S. "Aquitania" the economy in largely replacing expensive coal tar disinfectants, such as carbolic acid, cresol, etc., by electrolytic hypochlorite will approximately pay for the cost of the cell in the course of a single trip of three weeks.

THE OPTIMUM CONCENTRATION OF THE ELECTROLYTIC HYPOCHLORITE FOR DISINFECTANT PURPOSES IN WARDS, ETC. — It is a somewhat difficult matter to select the most advantageous concentration for the disinfecting fluid. As is well known, the hypochlorites possess extraordinarily high germicidal action when acting upon organisms suspended in pure water. One to two parts per million of hypochlorite will effectively kill, in two hours, a moderately large quantity of organisms other than spore forms. But this activity is much reduced when the hypochlorite acts in the presence of much extraneous matter. It is obvious, therefore, that the nature and quantity of the organic matter present on the soiled surfaces which are to be disinfected is a matter of importance. It would appear that a concentration of hypochlorite should be chosen, such as will insure an excess of hypochlorite on the treated area, for some short time after its application. This ideal concentration will of course vary with the nature of the surface treated and the amount of dirt upon it. Hypochlorite disappears rapidly when spread in a thin film on a wooden surface, less rapidly on linoleum, still less rapidly on rubber surfaces, while on a clean glass surface the hypochlorite may persist for thirty-six hours or more.

Practically speaking, it will be found that a strength of about 1 per 1000 sodium hypochlorite or available chlorine, when properly applied, will suffice for all ordinary purposes. On slightly contaminated smooth surfaces such as glass, rubber, and certain composition floors, 1 per 3000 or even less would probably be found sufficient, but no disadvantages follow the employment of the stronger solution. Electrolytic hypochlorite at a concentration of 1 per 1000 available chlorine can be used freely in the wards for swabbing and mopping the floors, walls, latrines, etc., without objectionable after-effect. This solution was conveniently obtained by taking the electrolyzed sea water through which the current had been passed for five minutes, previously described, and diluting each bucketful of this liquor with a bucketful of fresh salt water. The diluted mixture was kept in a large tub from which it was distributed to the wards.

The germicidal action of this mixture tested against typhoid organisms under the conditions of the Walker-Rideal test, may be calculated from the experiments of Klein, Sommerville and Walker, Rideal, and others. When diluted 20 times (*i.e.* a chlorine concentration of 1 : 20,000) it is as active as 1 per cent phenol.

Comparative experiments upon the bacteriological conditions of floors before treatment with salt water and after treatment with hypochlorite at 1 per 1000, showed a huge reduction in the number of organisms to follow the applications of hypochlorite, while a large reduction followed the use of plain sea water.

THE USES OF ELECTROLYTIC HYPOCHLORITE AS A DISINFECTANT IN WARDS, ETC. — The hypochlorite solution prepared as described in the previous section by mixing equal volumes of salt water electrolyzed for five minutes with plain salt water, was kept in large wooden tubs at convenient places near the wards on the different decks. Each morning after the floors had been brushed, a company of

sanitary orderlies, carrying the solution in wooden buckets, mopped every portion of the floor surface of the ward, under the beds, up the dividing walls, and in the lavatories.[1] On the wooden decks now enclosed and used for wards, the solution dries very quickly, while on linoleum, owing to its non-porous character, the wet surface takes longer to dry. It is desirable, therefore, that on linoleum a too large excess of liquid should not be left, or else a slippery moist surface persists for an inconveniently long time. It is important that the orderlies be instructed to change the disinfectant in their buckets at frequent intervals. The redistribution of dirt mixed with a little disinfectant of impaired activity over the surface of a ward does not constitute cleaning, and it is necessary that this is understood by the orderlies.

In the enteric and dysentery wards hypochlorite disinfectant was placed in the bed-pans before use and its marked deodorant action was much appreciated. After cleaning the bed-pans with a special spray, they were well rinsed with hypochlorite solution. At frequent intervals the disinfectant was used for mopping all the surfaces in the latrines, including the door-handles, and from time to time it was poured down the waste-pipes, followed by a good flush of salt water. When used in this way we have not observed damage greater than that caused by other disinfectants, as shown by the results of an actual examination of the plumbing in latrines where hypochlorite has been used compared with similar structures in latrines treated with phenol and other disinfectants. As is well known, intermittent use of electrolyzed sea water has been successfully employed on French naval ships to render urinal and water-closet traps unobjectionable (cf. Gatewood's Naval Hygiene, p. 443, Rebman, 1909).

[1] It may be of interest to mention the fact that the distance of corridors, floors, etc., mopped with the disinfectant exceeded three miles in the case of the Hospital Ship "Aquitania." In most cases it was possible to make one daily application only.

It has also been tried on British naval ships to a limited extent, but we have no details as to the concentration and quantity of hypochlorite used, and these are essential points.

It is worth noting that electrolytic hypochlorite can be used in conjunction with soap, and some particularly heavily soiled decks were cleaned with this mixture. It is probable, however, that reduction in the germicidal action of the hypochlorite is followed by the addition of soap. The hypochlorite was also used for putting in the spittoons of tuberculosis patients and for other similar purposes for which disinfectants are commonly employed.

The results of the free use of hypochlorite were excellent and the wards were fresher, cleaner, and freer from objectionable odor than they were previous to its use. In the typhoid and dysentery wards the results have been particularly striking and the absence of odor was most marked.

The most important effect of all has been the large reduction in secondary infection following upon the introduction of hypochlorite disinfection on large hospital ships. While it would be unfair to refer this fortunate result as exclusively due to the hypochlorite, it is generally considered among competent observers that the introduction of the hypochlorite has been an important cause of the improvement.

Rate of Decomposition of Electrolytic Hypochlorite on Keeping. — It is well known that hypochlorite solutions prepared by the direct electrolysis of sea water are unstable. For practical purposes of disinfection, etc., on shipboard, and in other places this is of no moment, since the solution is readily prepared as needed and there is no need for lengthy storage. On the average the rate of decomposition stored in open tubs was about twenty-five per cent of hypochlorite present in the twenty-four hours. The rate of decomposition is influenced by temperature and by free exposure to air. When stored in covered vats the rate of decomposition is materially less. Since the electrolytic hypochlorite is so

readily and cheaply prepared it is well to reject solutions which have been stored for more than two or three days.

The Action of Electrolytic Hypochlorite on Various Structural Materials. — Comparative experiments showed that clean iron and steel are attacked to a marked extent by 1 per 1000 hypochlorite, and the action is relatively rapid. Copper is much more slowly attacked, but the action is definite. Brass is still less attacked, while aluminium, zinc, nickel, and tin are scarcely attacked at all under the conditions of the experiments. Lead is the most resistant of all the metals tested. Tin plate, nickel plate, galvanized iron, are not appreciably attacked if the plating is intact. Organic materials such as wood, linoleum, rubber, composition floorings of various kinds, take up a certain amount of hypochlorite, as would be expected. Wood is the most active in this respect and rubber the least, but in no case is significant damage done to the material. Wooden tubs which have contained strong hypochlorite solutions for long periods develop a soft whitish deposit on the surface, but if this is not scraped off further action soon ceases. Painted wood and metal, at least so far as the materials tested were concerned, proved to be very resistant. None of the floor surfaces in the wards on board ship showed significant damage, the only complaint being, as already noted, that occasionally the saline hypochlorite dried rather slowly on damp days on the linoleum surfaces. But this is due to the non-absorbent character of the linoleum, especially when more or less saturated with salts from previous applications, rather than to any destructive action. These conditions can be easily remedied by occasional washing with fresh water.

It may be noted that sodium hypochlorite prepared in the apparatus described can be used successfully both for wound treatment and for the disinfection of drinking water. Details as to suitable concentrations for these purposes have already been given.

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